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Energy Metabolism in the Hypertrophied Heart

by

Brett Otto Schönekess ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirments for the degree of Doctor of Philosophy

Department of Pharmacology

Edmonton, Alberta
Spring 1996

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Faculty of Graduate Studies and Research

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Commitment

Commitment is what transforms a promise into reality.
It is the words that speak boldly of your intentions.
And the actions which speak louder than the words.
It is making the time when there is none.
Coming through time after time, year after year after year.
Commitment is the stuff character is made of;
the power to change the face of things.
It is the daily triumph of integrity over skepticism.

Anonymous.

Whether you think you can or think you can't,
you're right!

Henry Ford.

Change is good!

Anonymous.

Everyday is a good day to be alive!

BOS

Dedication

To my family and friends;
you know who you are.

Abstract

Introduction: Pressure-overload induced hypertrophy of the myocardium can lead to mechanical dysfunction, as well as an altered sensitivity to myocardial ischemia. The basis for these alterations may be linked to energy substrate metabolism. Changes in glycolytic enzyme content and isoenzyme profile, and deficiencies in essential co-factors for energy production are some proposed mechanisms regarded as important in the pathophysiology of the hypertrophied heart. To date, only a few studies of energy substrate use in the hypertrophied heart have been made, and most of these existing studies measured energy metabolism indirectly.

Purpose: The purpose of this thesis research was to directly determine the contribution of glucose, lactate, and fatty acids to energy production in hypertrophied rat hearts.

Methods: A pressure-overload cardiac hypertrophy was produced in juvenile male rats by placement of a hemoclip around the abdominal aorta. The natural growth of the animal over an 8 week period caused a gradual constriction of the abdominal aorta, resulting in the development of myocardial hypertrophy. Direct measurements of glycolysis, glucose, lactate, and palmitate oxidation were made in isolated working hearts.

Results: Initial findings suggested that the key metabolic alteration found in hypertrophied hearts was increased rates of glycolysis compared to normal hearts (3728 ± 549 (n=11) *vs* 2344 ± 226 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{g dry wt}^{-1}$ (n=9), respectively) . Rates of fatty acid oxidation were decreased, although this was dependent on mechanical work and the free fatty acid concentration in the perfusate. Acute loading of propionyl-L-carnitine (PLC) into hypertrophied hearts resulted in an improvement of mechanical function and increased rates of carbohydrate oxidation (glucose and lactate). Energy metabolism was also studied during and following ischemia in the hypertrophied heart. Following 30 min of ischemia, mechanical function was depressed in the hypertrophied heart compared to normal hearts, even though the recovery of ATP production was similar. During ischemia the severe depression of oxidative metabolism leaves glycolysis and glycogenolysis as major sources of ATP. Direct measurements of glycolysis and glycogenolysis during ischemia indicated that glycogen contributes significantly to glycolysis, but rates of glycolysis are not accelerated in the hypertrophied heart compared to normal hearts.

Conclusions: The hypertrophied heart's energy substrate profile is similar to that in normal hearts during ischemia and post-ischemic reperfusion. The major alteration is an acceleration of the rates of glycolysis during aerobic perfusion in the hypertrophied heart compared to normal hearts. Exaggerated post-ischemic mechanical depression seen in hypertrophied hearts, compared to normal hearts may be explained by accelerated rates of glycolysis uncoupled from glucose oxidation. This results in an increased H^+ production from the hydrolysis of glycolytically-derived ATP. This H^+ production may potentially lead to membrane ion imbalances and a decrease in cardiac efficiency.

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LIST OF ABBREVIATIONS

ADP:	adenosine diphosphate
AMP:	adenosine monophosphate
ATP:	adenosine triphosphate
CAT:	carnitine acetyltransferase
CPT I:	carnitine palmitoyltransferase I
CPT II:	carnitine palmitoyltransferase II
CoA:	coenzyme A
CP:	creatine phosphate
dP:	developed pressure
HR:	heart rate
LDH:	lactate dehydrogenase
NAD^+:	nicotinamide adenine nucleotide
NADH:	dihydronicotinamide adenine nucleotide
PCA:	perchloric acid
PDH:	pyruvate dehydrogenase
PLC:	propionyl-L-carnitine
PSP:	peak systolic pressure
TCA:	tricarboxylic acid cycle

CHAPTER I

Introduction

A) Myocardial hypertrophy

i) Definition and cause

Myocardial hypertrophy is an increase in the size and mass of cardiac myocytes. This may be due to a number of factors such as a genetic propensity for hypertrophic cardiomyopathy (Wigle et al., 1995), or in response to increased intra-ventricular pressure and the resultant wall stress (Grossman et al., 1975; Lorell 1987). Increased intra-ventricular pressure can occur due to a number of factors such as a pressure- or volume-overload of the circulatory system, from hypertension or fluid overload (Kannel et al., 1992; Armstrong and Moe 1994).

ii) Morbidity and mortality

A pressure-overload cardiac hypertrophy is a well recognized risk factor for the development of congestive heart failure (CHF), sudden death and myocardial infarction (Weber, 1988; Frohlich et al., 1992). In the hypertensive patient, myocardial hypertrophy (left-ventricular hypertrophy) is regarded as a sign of end-organ damage (Teo 1995), and is associated with an increased mortality and morbidity compared to the normal population (Rautaharju et al., 1988; Levy et al., 1994). Episodes of angina, myocardial infarction, and ventricular arrhythmias increase in patients with left ventricular hypertrophy. The effects of treating patients who have left-ventricular hypertrophy suggest that a regression of the hypertrophy may decrease the risk of developing cardiovascular disease in hypertensive subjects (Teo 1995).

iii) Underlying pathogenesis

Although the short term relief from elevated intra-ventricular pressures occurs with an increased ventricular mass and wall thickness, diastolic dysfunction can result (Hanrath et al., 1980; Eichorn et al., 1983; Topol et al., 1985; Lorell et al, 1986; Wexler et al., 1988). Myocardial hypertrophy resulting from severe hypertension is a an initial compensatory response, but eventually cardiac failure occurs due to a number of mechanisms (Braunwald et al 1976). Myocardial hypoperfusion may also exist due to an incongruent growth of myocardial mass compared to coronary vascularization and can lead to an impaired coronary reserve (Jeremy et al., 1989). A decreased subendocardial blood flow may contribute to increased rates of ischemic contracture or "stone heart" (Cooley et al., 1972; Hearse et al., 1977; Hutchins et al., 1979) in hypertrophied hearts compared to normal hearts (Attarian et al., 1981; Peyton et al., 1982). A depression in the recovery of mechanical function during post-ischemic reperfusion in hypertrophied myocardium compared to normal myocardium has been linked to increased rates of ischemic contracture (Snoeckx et al., 1986; Anderson et al., 1987).

Myocardial hypertrophy is associated with diastolic dysfunction and impairment of relaxation as well as alterations in myocardial contractility (Anderson et al., 1990a; Cunningham et al., 1990). An increased severity of these mechanical abnormalities is also seen during reperfusion of hypertrophied hearts following ischemia or hypoxia when compared to normal hearts (Menasche et al., 1985; Anderson et al., 1987, 1990a; Cunningham et al., 1990; Gaasch et al., 1990). The hypertrophied heart undergoes a fundamental change, in that the duration of isometric contraction and relaxation is increased possibly due to a parallel shift in myosin isoenzymes from a faster (V_1) to slower (V_3) enzymatic form (Mercasdier et al., 1981). This shift in isoenzyme pattern reflects a reduction in myofibrillar ATPase activity, and a decrease in shortening velocity (Jacob et al., 1986).

Deficiencies in the mechanisms responsible for the handling of intracellular Ca^{2+} exist in the hypertrophied heart (Gwathmey and Morgan, 1985; Bentivegan et al., 1991; Bailey and Houser, 1993; Perreault et al., 1993). There may also be a greater dependence on Na^+/Ca^{2+} exchange as a regulatory means of maintaining intracellular Ca^{2+} (Pernollet et al., 1981; Sharma et al., 1986; Nakanishi et al, 1989). As well it has been shown that an increased Ca^{2+} accumulation occurs in the hypertrophied heart during post-ischemic reperfusion compared to normal hearts (Allard et al., 1994b). Hypertensive rats which also show myocardial hypertrophy, also have a greater Ca^{2+} accumulation compared to normal hearts during post-ischemic reperfusion (Nayler, 1983). There is also evidence of increased Na^+ accumulation in the hypertrophied heart which may be linked to ischemic dysfunction (Anderson et al., 1990b; Mochizuki et al., 1993; Golden et al., 1994). A lower activity of the sarcolemmal Na^+/K^+ ATPase has also been reported in the hypertrophied heart (Pernollet et al., 1981; Lee et al., 1983; Sharma et al., 1986; Whitmer et al., 1986; Nakanishi et al, 1989). Acidosis itself has not been linked to hypertrophied hearts during normal aerobic perfusion per se, but is associated with a decrease in twitch tension during ischemia and hypoxia (Ricciardi et al., 1986; Lee and Allen, 1991). However, myocardial acidosis does not influence the contractile apparatus differently in the hypertrophied heart compared to normal hearts (Mayoux et al., 1994). All of these deficiencies in ion handling may lead to ionic imbalances that could potentially be a link to increased susceptibility and prevalence of the hypertrophied heart to arrhythmias (Avkiran, 1994).

The hypertrophied heart is susceptible to myocardial ischemia, and has been found to have a depressed mechanical function upon post-ischemic reperfusion when compared to normal hearts (Snoeckx et al., 1986; Anderson et al., 1987; Buser et al., 1990). The changes in the hypertrophied myocardium responsible for the increased susceptibility to ischemia and the mechanical dysfunction has received considerable research attention. An alteration in myocardial energy substrate use by the hypertrophied heart has been implicated as an important contributory factor in the pathophysiology of cardiac hypertrophy (Bishop and Aultschuld, 1971; Buser et al., 1990), and the enhanced deterioration of mechanical function in the hypertrophied heart (Menasche et al., 1985; Anderson et al., 1987, 1990a; Cunningham et al., 1990). Overall oxygen consumption may be decreased in the hypertrophied heart (Anderson et al., 1988, 1989), suggesting alterations in energy metabolism. Nishio et al., (1995) have recently shown that a moderate pressure overload stimulus leading to cardiac hypertrophy results in a rate of mitochondrial biogenesis sufficient to maintain a constant mitochondrial content relative to tissue mass, therefore mitochondrial content is not affected. However, no definitive studies looking directly at energy substrate metabolism have been carried out in the hypertrophied heart during normal aerobic conditions or during ischemia and post-ischemic reperfusion.

B) Mammalian heart and its primary source of energy

i) Aerobic production of ATP

The mammalian heart can use a variety of carbon substrates for the production of ATP. Fatty acids, lactate, glucose, and to a lesser extent ketone bodies and amino acids, can all be metabolized to produce ATP (Neely and Morgan, 1974; Saddik and Lopaschuk 1991, van der Vusse et al., 1992ab). The extent to which these exogenous sources contribute to ATP production varies, and depends on the availability and presence of competing carbon substrates. However, the oxidation of fatty acids is known to be the primary source for the reducing equivalents required to produce ATP in the heart, and the majority of the heart's energy needs are met through the oxidation of fatty acids (Neely and Morgan, 1974; Saddik and Lopaschuk, 1991). The oxidation of glucose and lactate provide most of the remaining energy needs for the heart, with glycolysis providing an additional small amount of ATP (Saddik and Lopaschuk, 1991). Controversy exists as to the contribution that lactate plays in providing the heart with ATP. Lactate has been suggested to be the primary source of ATP production in the heart (Drake et al., 1980), as lactate can contribute substantially to ATP production under extreme circumstances (Spitzer and Spitzer 1972; Scott et al., 1972; Spitzer 1974), and during exercise (Stanley, 1991; Gertz et al., 1988). It has been suggested that an inverse relationship exists between lactate and fatty acid use (Drake et al., 1980; Bielefeld et al., 1985). However, no definitive studies showing the exact contribution of lactate and fatty acids to overall ATP production have been performed.

The endogenous triacylglycerol pool is an important source of fatty acids for oxidative metabolism (Saddik and Lopaschuk, 1991,1992). Triacylglycerol can contribute anywhere from 10-50% of the ATP produced in the isolated perfused rat heart (Saddik and Lopaschuk, 1991). The exact contribution is dependent on the amount of exogenous free fatty acids in the perfusion buffer. Glycogen is another source of ATP in the heart. The contribution of glycogen to aerobic ATP production has recently been found to be substantial (about 40% of the ATP derived from glucose metabolism) (Henning et al., 1995; Goodwin et al., 1995). Therefore it is evident that endogenous carbon sources such as triacylglycerol and glycogen contribute to ATP production.

ii) Sources of carbon substrate used for ATP production

a) Fatty acids

Fatty acids are present in the blood either as unesterified molecules (free fatty acids) or as esterified mono, di, or triacylglycerols, phospholipid or cholesterol esters. Albumin is a carrier protein in blood plasma which increases the solubility of free fatty acids. Fatty acid esters, i.e. triacylglycerols, are carried in the blood by carrier molecules. The core of these carrier molecules is composed of triacylglycerols, and the surrounding shell is composed of phospholipids, cholesterol, and apoproteins (Dolphin, 1985). Chylomicrons are triacylglycerol-rich particles formed by epithelial cells of the small intestine. Very low-density lipoprotein (VLDL) particles are also a source of fatty acids for the heart. These particles are synthesized in parenchymal liver cells (Spector, 1984). Once in the coronary circulation, fatty acids are released from chylomicrons and VLDL particles by the action of a vascular endothelium-bound enzyme, lipoprotein lipase (LPL) (Cryer, 1989). The fatty acids released by this enzyme in from chylomicrons and VLDL particles, bind to albumin and are transported into the endothelial cell, and into the myocyte where they are used primarily as an energy substrate.

The mechanism by which the fatty acids pass into the myocyte is not clearly defined. A membrane-spanning fatty acid transport protein has been identified in adipocytes, and a similar mRNA for this transporter also exists in heart muscle (Schaffer and Lodish, 1994). This transport mechanism would be required in the luminal and abluminal membrane of the coronary endothelial cells, as well as the plasma membrane of the myocytes to ensure transport across all three membranes. Free fatty acids that pass through the coronary endothelial cells and pass into the interstitial space are solubilized by binding to albumin. After being transported through the plasma membrane of the myocyte (Schaffer and Lodish, 1994), intracellular transport is believed to occur by fatty acids binding to fatty acid binding proteins (FABP) which are abundant in myocytes (van der Vusse et al., 1992b), and may also exist in the endothelial cells. However, van Nieuwenhoven et al., (1995) have found that myocardial endothelial cells show no significant fatty acid binding activity, and only minor quantities of cytoplasmic FABP. This lead the authors to speculate that fatty acid transport across the endothelial cytoplasm occurs via aqueous diffusion and not in a carrier mediated fashion.

Once inside the myocyte, fatty acids must enter the mitochondria to undergo ß-oxidation. The activation of long-chain fatty acids to long-chain fatty acyl-CoA esters occurs in the myocyte via an energy dependent process catalyzed by long-chain fatty acyl-CoA synthetase, an outer mitochondrial membrane bound protein (Normann et al., 1983; Schulz, 1994). Activated long-chain fatty acyl-CoA can also be used for triacylglycerol synthesis in the heart. Fatty acyl-CoA not shunted towards triacylglycerol synthesis is transported into the mitochondria. This is achieved by a carnitine-mediated translocation involving carnitine palmitoyltransferase I (CPT I), carnitine acyltranslocase, and carnitine palmitoyltransferase II (CPT II). By virtue of its role in the translocation of fatty acids into the mitochondria, L-carnitine is essential for the ß-oxidation of long-chain fatty acids. Upon entry into the mitochondrial matrix, fatty acids undergo ß-oxidation to yield acetyl-CoA, NADH and H^+, and $FADH_2$. Acetyl-CoA enters the tricarboxylic acid (TCA) cycle to further produce reducing equivalents for the electron transport chain, and ATP is subsequently produced.

b) Glucose, lactate and pyruvate

The other major energy substrates for the heart are carbohydrates. Glucose, is transported into myocytes by a facilitative transporter (GLUT 1) which may be responsible for basal glucose uptake. Insulin increases glucose transport by stimulating the translocation of the GLUT 4 transporter from an intracellular pool to the plasma membrane (Simpson and Cushman, 1986; Pessin and Bell, 1992; Gould and Holman, 1993). Contractile work and β-stimulation have a similar effect on glucose transport. Once inside the myocyte, glucose is phosphorylated by hexose kinase and can then be shunted to glycogen synthesis or shuttled through glucose phosphate isomerase. Glycogen synthesis is supported in hearts which are exposed to non-carbohydrates sources of energy, such as free fatty acids (Laughlin et al., 1994). Glucose metabolism can be separated into two pathways. The first pathway involves the anaerobic catalysis (glycolysis) of one glucose molecule to two pyruvate molecules (Embden-Meyerhof pathway). The rate-limiting step of this pathway during aerobic perfusion of the heart is catalyzed by the enzyme phosphofructokinase (PFK). During situations where oxygen supply is limited (hypoxia or ischemia) the enzyme glyceraldehyde 3-phosphate dehydrogenase becomes rate limiting (Taegtmeyer, 1985). Pyruvate from glycolysis can pass into a second pathway by being metabolised by pyruvate dehydrogenase (PDH) to form acetyl-CoA for the TCA cycle. Alternatively pyruvate can be reduced by NADH and form lactate, or be transaminated to form alanine. For every glucose molecule that leads to two pyruvate molecules, 2 ATP will be formed from glycolysis, and an additional 36 molecules of ATP upon complete oxidation (glucose oxidation).

Lactate and pyruvate can be transported into the myocyte via a monocarboxylic acid transporter (Poole and Halestrap, 1993). Pyruvate can immediately be oxidized by the PDH complex and enter the TCA cycle to produce reducing equivalents for ATP production (NADH and $FADH_2$). Lactate must first be oxidized by lactate dehydrogenase (LDH) to form pyruvate and NADH, before it can be utilized for further energy production. The exact contribution of lactate to overall ATP production in the heart is a matter of controversy.

c) Endogenous triacylglycerol and glycogen

Long-chain fatty acyl groups are found as a storage form in the myocardium esterified to glycerol. The importance of triacylglycerols, for ATP production has been shown to be significant (Saddik and Lopaschuk, 1991). However, the contribution of triacylglycerol is dependent on the availability of exogenous fatty acids. Increased levels of exogenous fatty acids may stimulate the production of triacylglycerol in the heart via the activation of phosphatidic acid phosphohydrolase, and by the inhibition of triacylglycerol lipolysis (Schoonderwoerd et al., 1989).

A storage form of glucose in the myocardium is glycogen. Glycogen is a large molecule with a branched structure and a molecular weight of about 10^7 Da (Alonso et al., 1995). The control of glycogen metabolism is complex in that the synthetic pathway (involving the enzyme glycogen synthase) and the degradative pathway (glycogen phosphorylase) are separate. The regulation of these pathways occurs in a reciprocal manner, where one is stimulated and the other is inhibited (Williams and Mayer, 1966; Neely and Morgan, 1974). The heart has a great potential to increase its store of glucose in the form of glycogen. This phenomenon, known as supercompensation, can be seen in rat hearts during starvation (Adrouny et al., 1956) and after exercise (Gaesser and Brooks, 1980).

iii) ATP production during ischemia

A severe ischemia results in a shift in energy production away from oxidative metabolism towards the anaerobic production of ATP via glycolysis. The increased ratio of NADH/NAD$^+$ due to the lack of O_2 leads to a suppression of β-oxidation (Liedtke, 1981). During low-flow ischemia, where limited O_2 is available for oxidative phosphorylation, glycolysis is accelerated but fatty acids represent the major residual substrate used for oxidative metabolism (Liedtke et al., 1975; Fox et al., 1983). The source of glucose for glycolysis during low-flow ischemia is exogenous glucose as well as glycogen, with glycogen contributing the majority of glucose for glycolysis during no-flow ischemia. The breakdown of glycogen during ischemia is initiated by the allosteric stimulation of phosphorylase *b* due to increased levels of AMP and P_i, and decreased levels of ATP and glucose-6-phosphate (Neely et al., 1970; Neely and Morgan, 1974; Taegtmeyer 1985). The contribution of glycogen to glycolysis or residual glucose oxidation during ischemia has not previously been investigated by direct measures.

iv) ATP production during post-ischemic reperfusion

Plasma fatty acid levels increase following an acute myocardial infarction (Oliver et al., 1968; Allison et al., 1969; Opie, 1975) or during cardiac surgery (Lopaschuk et al., 1994), such that during post-ischemic reperfusion the myocardium is exposed to high concentrations of fatty acids. The detrimental effects of high plasma fatty acid levels, combined with increased concentrations of catecholamines, on mechanical and electrophysiological characteristics of the heart following post-ischemic reperfusion has been recognized for a number of years (Opie, 1975). The primary source of ATP during post-ischemic reperfusion has been found to result from the oxidation of exogenous and endogenous fatty acids (Saddik and Lopaschuk, 1992). The recovery of oxidative metabolism occurs rapidly in the mammalian heart during post-ischemic reperfusion (Liedtke et al, 1988; Lopaschuk et al., 1990; Görge et al., 1991; Saddik and Lopaschuk, 1992).

C) Metabolic alterations in myocardial hypertrophy

i) Fundamental changes in metabolic processes

Pressure-overload cardiomyopathies are associated with changes in the isoenzyme pattern of LDH from the aerobic form to the more anaerobic or fetal-like form (Taegtmeyer and Overturf, 1988). There are also changes in creatine kinase (CK), in that the isoenzyme pattern reverts to a fetal like form (i.e., from the CK-MM to the CK-MB form) (Bishop and Aultschuld, 1971; Ingwall, 1984; Michelletti et al., 1994a). Tissue L-carnitine, important for the transport of long-chain fatty acids into the mitochondrial matrix, is also decreased in the hypertrophied heart (Reibel et al., 1983; El Alaoui-Talibi et al., 1992). These alterations, as well as other changes in myocardial energy metabolism, have been implicated as contributing to the depression in post-ischemic contractile function (Bishop and Aultschuld, 1971; Reibel et al., 1983; Ingwall, 1984; Taegtmeyer and Overturf, 1988; Cunningham et al., 1990; Eberli et al., 1991; El Alaoui-Talibi et al., 1992; Schwartz et al., 1992; Yang et al., 1992; Cheikh et al., 1994; Michelletti et al., 1994b).

ii) Aerobic production of ATP

Despite the changes listed above, the actual contribution of the various metabolic pathways to aerobic ATP production in hypertrophied hearts has not been determined. What has been reported is that fatty acid oxidation is decreased in hypertrophied hearts due to volume-overload exists (El Alaoui-Talibi et al., 1992; Cheikh et al., 1994). Also, the oxidation of fatty acids is reduced in hearts from guinea pigs with aortic constriction (Weber, 1988). There exists the possibility that a limitation in carbon substrate use, specifically from long-chain fatty acids such as palmitate occurs in hypertrophied hearts (Cheikh et al., 1994). Severely hypertrophied hearts (a 66% increase in dry heart weight of hypertrophied hearts compared to controls) exhibit a loss of respiratory control and this appears to be due to a limitation in the availability of appropriate oxidizable substrate. A loss of the relationship between the cytosolic adenylate system (i.e., control of respiration by ADP) and myocardial oxygen consumption supports the hypothesis that the hypertrophied heart may be limited in mitochondrial NADH, especially during high work periods.

The reason for decreased rates of fatty acid oxidation in hypertrophied hearts is not clear, but has been suggested to occur secondary to the decreased L-carnitine content seen in hypertrophied myocardium (Wittels and Spann, 1968; Whitmer, 1986; Reibel et al., 1983; El Alaoui-Talibi et al., 1992). L-carnitine is a required co-factor for the translocation of long chain fatty acids into the mitochondria where β-oxidation occurs. Since fatty acid oxidation is the primary source of ATP production in the heart, a decrease in fatty acid oxidation may result in a depression of contractile function secondary to a decrease in ATP production.

iii) ATP production during ischemia

An altered energy metabolism in hypertrophied hearts has important implications in the setting of myocardial ischemia. Previous studies have suggested that lactate production due to anaerobic glycolysis (a major source of ATP during myocardial ischemia) in hypertrophied hearts increases both during hypoxia (Bishop and Aultschuld, 1971; Anderson et al., 1990a) and ischemia (Anderson et al., 1990a). It has also been suggested that the hypertrophied heart may have decreased glycolytic rates during and following hypoxia (Cunningham et al., 1990) with the effect of impairing diastolic function (Lorell et al., 1986; Wexler et al., 1988). In failure, the hypertrophied heart may have a reduced capacity to recruit anaerobic glycolysis, and this may contribute to diastolic dysfunction during ischemia and subsequent post-ischemic reperfusion (Gaasch et al., 1990). Contrary to this evidence for alteread rates of glycolysis in the hypertrophied heart, is evidence put forward by Massie et al., (1995) which shows that hypertrophied pig hearts does not have an altered basal rate of glucose metabolism, but has a increased ability to utilize glucose via both glycolysis and glucose oxidation upon dobutamine stress. The potentially altered glycolysis may be linked to the modulation of some key glycolytic enzymes, such as CK, LDH, and PFK (Ingwall, 1984; Taegtmeyer and Overturf, 1988). Controversy therefore exists as to whether or not the hypertrophied heart is able to recruit anaerobic glycolysis during hypoxia or post-ischemic reperfusion (Cunningham et al., 1990; Gaasch et al., 1990; Anderson et al., 1990a).

iv) ATP production during post-ischemic reperfusion

In hypertrophied hearts there is an increased susceptibility to ischemia-induced injury relative to that seen in normal hearts (Hearse et al., 1978; Anderson et al., 1990ab; Buser et al., 1990). The recovery of oxidative metabolism in the heart is critical for mechanical function following myocardial ischemia. In non-hypertrophied hearts, fatty acid oxidation predominates as a source of energy during post-ischemic reperfusion, with overall oxidative metabolism quickly recovering following short periods of ischemia (Lopaschuk et al., 1990). In hypertrophied hearts fatty acid oxidation can be depressed even in the absence of ischemia, possibly due to a decrease in myocardial L-carnitine content (Wittels and Spann, 1968; Whitmer, 1986; El Alaoui-Talibi et al., 1992; Cheikh et al., 1994). This may lead to a decreased energy reserve in the hypertrophied heart (Ingwall, 1984). With the observation by Cheikh et al (1994) that the hypertrophied heart is possibly in a state of energy starvation, it is unclear whether or not the recovery of carbohydrate or fatty acid oxidation in the hypertrophied heart is compromised during post-ischemic reperfusion.

A number of studies have suggested that a rapid recovery of glycolysis is also necessary for the recovery of mechanical function in the normal heart during post-ischemic reperfusion (Opie 1990; Eberli et al., 1991; Jeremy et al., 1992; du Toit and Opie 1992; Vanoverschelde et al., 1994). However, with the controversy that exists as to whether or not the hypertrophied heart is able to recruit anaerobic glycolysis during post-ischemic reperfusion (Anderson et al., 1990a; Cunningham et al., 1990; Gaasch et al., 1990), there has been no definitive studies done to directly determine what happens to glycolysis during post-ischemic reperfusion.

D) Optimizing energy metabolism in the heart

i) Effects of myocardial L-carnitine deficiency on oxidative metabolism

Alterations in the metabolism of fatty acids can occur when perturbations such as tissue L-carnitine depletion occur (El Alaoui-Talibi et al., 1992). These perturbations can lead to impairment of myocardial function (Siliprandi et al., 1987). Most known situations associated with myocardial L-carnitine deficiencies are also associated with a depression in myocardial function, i.e., myocardial hypertrophy and diabetes. Whether fatty acid oxidation rates are depressed in L-carnitine-deficient hearts probably depends to a large extent on the severity of the L-carnitine deficiency, as well as on the presence of other carbon substrates.

In primary and secondary L-carnitine deficiencies the depressed myocardial function is presumed to occur secondary to a depression of fatty acid oxidation (Table 1-1). Experimentally-induced L-carnitine deficiencies, such as that following sodium pivalate treatment of rats, can result in a 50-60% reduction in myocardial L-carnitine content. This severe L-carnitine deficiency results in a depression of cardiac function when the treatment is extended for periods of 24-26 wk. These results suggest that in severe L-carnitine deficiencies, CPT I activity is inhibited resulting in a decrease in fatty acid oxidation (Broderick et al., 1995a).

Accompanying the decreased rates of fatty acid oxidation in sodium pivalate treated hearts is an increase in glucose oxidation rates (Broderick et al., 1995a). An increase in glucose oxidation in L-carnitine deficient hearts would appear to contradict the observations that L-carnitine supplementation to normal hearts also increases glucose oxidation rates (Broderick et al., 1992). However, these apparent contradictions can readily be explained by the importance of intramitochondrial acetyl-CoA/CoA levels in regulating glucose oxidation. In severe L-carnitine deficiencies where fatty acid oxidation is inhibited, acetyl-CoA supply from β-oxidation will decrease. This will decrease the ratio of intramitochondrial acetyl-CoA/CoA, relieving the inhibition of PDH. The end result is that the activity of PDH will increase and rates of glucose oxidation will accelerate. In normal hearts where fatty acid oxidation rates are not depressed, the effects of L-carnitine on the intramitochondrial acetyl-CoA/CoA ratio would be expected to parallel what is seen in a severe L-carnitine deficiency, resulting a similar increase in glucose oxidation. As a result, L-carnitine deficiency and its affect on glucose oxidation probably depends on whether the deficiency is severe enough to inhibit fatty acid oxidation.

ii) Potential therapies to treat metabolic deficiencies in the heart

L-carnitine and propionyl L-carnitine (PLC), a naturally occurring derivative of L-carnitine, can effectively increase tissue L-carnitine levels (Paulson et al., 1986). Both compounds have previously been shown to improve heart function in pathological conditions such as diabetes and myocardial hypertrophy (Paulson et al., 1984; Paulson et al. 1986; Siliprandi et al., 1987; Liedtke, et al., 1988b; Motterlini et al., 1992). L-carnitine may be a potential candidate as a regulator of both carbohydrate and fatty acid oxidation. In normal hearts, L-carnitine pre-treatment results in a dramatic increase in the contribution of carbohydrate oxidation to ATP production (Table 1-2). In diabetic rat hearts, where fatty acid oxidation provides almost all of the ATP requirements (Garland et al., 1964; Randle, 1986; Wall and Lopaschuk 1989; Broderick et al., 1995b), L-carnitine markedly increases rates of glucose oxidation. Studies involving hypertrophied hearts and PLC have suggested that PLC will improve the energy state of hypertrophied hearts (Motterlini et al., 1992; Yang et al., 1992), which is associated with increased fatty acid oxidation. However, PLC may affect carbohydrate metabolism in a similar manner as L-carnitine; i.e., by increasing carbohydrate oxidation (Moravec et al., 1995).

Therefore potential therapies aimed at reversing the metabolic alterations in the hypertrophied heart have focused on trying to improve the deficiencies seen in L-carnitine content by supplementing the heart with L-carnitine or L-carnitine derivatives (Table 1-2). Beneficial effects of PLC on functional and hemodynamic parameters have been shown in both *in vivo* and *in vitro* animal studies (Motterlini et al., 1992; Yang et al., 1992; Michelletti et al., 1994a), and in humans (Bartels et al., 1992). As well, PLC will improve the mechanical function of both normal and hypertrophied hearts following ischemia (Paulson et al., 1986; Liedtke et al., 1988b).

a) Mechanism of action of L-carnitine and L-carnitine derivatives

While the beneficial effects of PLC are thought to occur via improvement of fatty acid oxidation, it cannot be discounted that the beneficial effects of PLC may also occur secondary to a stimulation of glucose oxidation. It has been demonstrated that L-carnitine has an important role in regulating carbohydrate oxidation (Broderick et al, 1992, 1993). This may occur due to L-carnitine's ability to also transport acetyl groups from within the mitochondrial matrix to the cytosol (Pearson and Tubbs, 1967; Lysiak et al., 1986, 1988). Carnitine acetyltransferase catalyses the transfer of acetyl groups from acetyl-CoA to L-carnitine forming acetylcarnitine. The acetylcarnitine can then be transported into the cytosol, where the acetyl groups are transferred back onto CoA. Recent interest has stemmed from this proposed role of L-carnitine as a modulator of the intramitochondrial acetyl-CoA/CoA ratio (Lysiak et al., 1986, 1988; Broderick et al., 1992). In isolated heart mitochondria, L-carnitine has been shown to increase CoA levels and reduce acetyl-CoA levels, resulting in a 10- to 20-fold decrease in the ratio of acetyl-CoA/CoA (Lysiak et al., 1986, 1988). In human skeletal muscle mitochondria this decrease in acetyl-CoA/CoA stimulates pyruvate oxidation, secondary to an increase in PDH activity (Uziel et al., 1988). Changes in the ratio of acetyl-CoA/CoA in the presence of L-carnitine are associated with an increased efflux of acetylcarnitine from the mitochondria (Lysiak et al., 1988), which is consistent with the suggestion that L-carnitine increases the activity of the carnitine acetyltransferase present on mitochondrial membranes.

Of interest is the observation that this L-carnitine-induced increase in glucose oxidation is accompanied by a concomitant decrease in fatty acid oxidation rates, such that overall ATP production rates remain similar to those in hearts not supplemented with

L-carnitine (Broderick et al., 1992). While this effect of L-carnitine on fatty acid oxidation would appear paradoxical, it is not if one considers that the primary role of L-carnitine is to ensure an adequate supply of acetyl-CoA for the TCA cycle. As shown in Figure 1-1, L-carnitine has a critical role in regulating the supply of acetyl-CoA from both PDH and from β-oxidation of fatty acids. Since the primary supply of acetyl-CoA is normally fatty acid ß-oxidation, an increase in TCA cycle activity (e.g., by increasing myocardial workload) will increase the supply of acetyl-CoA derived from fatty acid ß-oxidation (Neely and Morgan, 1974). An adequate myocardial level of L-carnitine is required to ensure that fatty acid ß-oxidation is able to meet mitochondrial acetyl-CoA demand. Provided that intramitochondrial acetyl-CoA supply from fatty acid ß-oxidation is not limited, the primary effect of L-carnitine supplementation is to regulate the supply of TCA cycle acetyl-CoA that is derived from PDH. By shuttling intramitochondrial acetyl-CoA out of the mitochondria and into the cytosol, via the carnitine acetyltransferase and carnitine acetyltranslocase pathway, intramitochondrial levels of acetyl-CoA will decrease. The lower acetyl-CoA/CoA ratio will result in a stimulation of PDH activity (Patel and Roche, 1990). This, in turn, will result in increased rates of glucose oxidation (Broderick et al., 1992). The need for ATP at a given workload is constant assuming adequate carbon substrate availability and oxygen supply. Therefore, an increase in acetyl-CoA derived from PDH would be expected to result in a decrease in the requirements of acetyl-CoA derived from β-oxidation. This would explain the observed decrease in myocardial fatty acid oxidation that accompanies the increase in glucose oxidation following L-carnitine supplementation to isolated perfused hearts

(Broderick et al., 1992).

It is clear that the role of L-carnitine is complex in its regulation of fatty acid and carbohydrate metabolism. In severe tissue L-carnitine deficiencies, the effects of L-carnitine supplementation on overall myocardial metabolism differs from the effects of L-carnitine supplementation when a L-carnitine deficiency does not exist. Whether the primary effect of L-carnitine is to stimulate fatty acid oxidation or glucose oxidation depends on the intramitochondrial acetyl-CoA/CoA ratio.

As discussed, L-carnitine supplementation to intact hearts increases glucose oxidation and decreases fatty acid oxidation, such that overall ATP production is maintained (Broderick et al., 1992). As shown in Figure 1-1, the effects of L-carnitine on glucose oxidation can be explained by a decrease in the intramitochondrial acetyl-CoA/CoA ratio. However, the effects of L-carnitine supplementation on fatty acid oxidation are less obvious, since a decrease in the acetyl-CoA/CoA ratio should also act as a stimulus to increase β-oxidation of fatty acids. Increasing both glucose and fatty acid oxidation at a given myocardial workload would decrease myocardial efficiency since ATP production (and O_2 consumption) would increase in the absence of additional demands for ATP.

b) Potential anaplerotic effect of PLC

PLC may also be beneficial by replenishing TCA cycle intermediates, thereby increasing overall TCA cycle pool size (Peuhkurinen 1982; Sundqvist et al., 1984; Di

Lisa et al., 1994). The mechanism by which PLC potentially increases TCA pool size is via PLC's ability to act as an effective anaplerotic substrate for the synthesis of succinyl CoA (Davies et al., 1980; Di Lisa et al., 1989, 1994; Tassani et al., 1994). PLC addition to ischemic and post-ischemic reperfused heart has been shown to be of benefit on post-ischemic mechanical function, as well as metabolic parameters such as high energy phosphate content (Paulson et al., 1986; Liedtke et al., 1988b; Sassen et al., 1991; Siliprandi et al., 1991). However a recent report by Sundqvist et al. (1994) suggests that PLC does not have any beneficial effect on the mechanical function of post-ischemic myocardium.

c) Effects of L-carnitine supplementation on myocardial oxidative metabolism and contractile function of hypertrophied hearts

The supplementation of the myocardium with L-carnitine or PLC results in an increased tissue L-carnitine content, and improves the recovery of hypertrophied heart function during reperfusion (Folts et al., 1978; Liedtke and Nellis, 1979; Hulsmann et al., 1985; Paulson et al., 1986; Motterlini et al., 1992; Yang et al., 1992; Broderick et al., 1993). The effects of L-carnitine or PLC administration in a variety of pathologies, including myocardial hypertrophy, have been shown to be beneficial (Table 1-3). Accumulating evidence suggests that the mechanism behind the beneficial effect of L-carnitine and PLC is not always via an increased rate of fatty acid oxidation or by decreasing the levels of potentially toxic levels of long-chain acyl-CoA. L-carnitine-mediated increases in the rates of carbohydrate metabolism (glucose and lactate oxidation) (Broderick et al., 1992, 1993) provide an alternate mechanism by which L-carnitine and PLC exert their beneficial effects.

d) Benefits of increasing carbohydrate oxidation by L-carnitine supplementation

High levels of fatty acids have a detrimental effect on post-ischemic mechanical recovery of hearts subjected to a severe episode of ischemia (Wall and Lopaschuk, 1989; Lopaschuk et al., 1990). While the exact mechanism by which fatty acid oxidation contributes to ischemic injury is not clear, our studies suggest that this may be related to their ability to inhibit glucose oxidation (Lopaschuk et al., 1988,1990,1993; McVeigh and Lopaschuk, 1990). High levels of fatty acid increase the intramitochondrial acetyl-CoA/CoA and NADH/NAD$^+$ ratios (Patel and Roche, 1990), which in turn inhibits PDH through the activation of a pyruvate dehydrogenase kinase (Patel and Roche, 1990; Kerbey et al., 1985). This inhibition of glucose oxidation during reperfusion can lead to a substantial imbalance between glycolysis and glucose oxidation during the actual reperfusion period (Lopaschuk et al., 1993). The result is an increase in the production of H$^+$ formed by the hydrolysis of glycolytically-derived ATP. It is this imbalance and exaggerated production of H$^+$ that may be mediating the detrimental effects of high levels of fatty acids on the recovery of post-ischemic mechanical function. The production of H$^+$ during ischemia and early in post-ischemic reperfusion could lead to increased activity of the Na^+/H^+ and the Na^+/Ca^{2+}-exchangers and result in a potentially damaging Ca^{2+} overload (Tani and Neely, 1989; Tani, 1990).

L-carnitine and PLC can improve functional recovery of normal and hypertrophied hearts reperfused following a severe episode of ischemia (Paulson et al., 1986; Liedtke et al., 1988b; Broderick et al., 1993). The actions of L-carnitine and PLC cannot be explained secondary to a stimulation of fatty acid oxidation during post-ischemic reperfusion. This is because fatty acid oxidation rates are not depressed during the post-ischemic reperfusion of normal hearts. In fact, due to the high circulating levels of fatty acids normally seen during reperfusion (Oliver et al., 1968), fatty acid oxidation provides over 90% of ATP production during reperfusion (Lopaschuk et al., 1990; Liedtke et al., 1988a; Görge et al., 1991; Saddik and Lopaschuk, 1992). In the presence of high levels of fatty acids, glucose oxidation provides only 5 to 10% of the ATP requirements. As previously mentioned, if glucose oxidation is stimulated during reperfusion the detrimental effect of high levels of fatty acids on depressing post-ischemic mechanical function can be overcome. Compounds which stimulate glucose oxidation directly, such as dichloroacetate (McVeigh and Lopaschuk, 1990; Lopaschuk et al., 1993), or indirectly such as CPT I inhibitors (Lopaschuk et al., 1988, 1993) improve the recovery of post-ischemic mechanical function. Recent evidence shows that the beneficial effects of L-carnitine on the recovery of post-ischemic mechanical function are associated with a marked increase in glucose oxidation (Broderick et al., 1993).

E) Energy substrate metabolism in the hypertrophied heart.

It is clear that the metabolic fate of exogenous and endogenous energy substrates used in the production of ATP in the hypertrophied heart is an area of unresolved importance. Many studies acknowledge that the hypertrophied heart has a potentially altered metabolic substrate use, but this has been done mostly by indirect methods such as measuring glycolysis by looking at lactate release, or simply looking at high energy phosphate content. The number of different interpretations of the hypertrophied heart's capacity for glycolysis, ability to recruit glycolysis, or the actual rates of glycolysis during hypoxia, ischemia, and post-ischemic reperfusion is confusing. Additionally an altered ability of the hypertrophied heart to oxidize fatty acids has been linked to the depressed myocardial tissue content of L-carnitine, an essential factor for the translocation of activated long-chain fatty acids into the mitochondria. There have been no reports measuring rates of carbohydrate oxidation in the hypertrophied heart. Thus, no clear information on the hypertrophied heart's ability to metabolize energy substrates for ATP production during either normal aerobic conditions or following an ischemic episode i.e., during post-ischemic reperfusion, exists.

Therefore, the focus of this thesis was to investigate energy substrate metabolism in pressure-overloaded hypertrophied heart. Specifically, investigations focused on:

1) Whether or not the hypertrophied heart is deficient in its ability to metabolize exogenous fatty acids;

2) Whether or not glycolysis and/or oxidative metabolism are altered during normal

aerobic perfusion and/or post-ischemic reperfusion;

3) Is there is a metabolic link to the mechanical depression seen in hypertrophied hearts during post-ischemic reperfusion?;

4) Can aerobic and post-ischemic mechanical function be improved by overcoming the L-carnitine deficiency seen in hypertrophied hearts and if so what are the effects on oxidative metabolism?;

5) Is the hypertrophied heart able to recruit glycolysis during ischemia and what is the contribution of glycogenolysis to ATP production during ischemia?;

6) Additionally, a study on the contribution of lactate and fatty acids to overall ATP production in normal hearts was performed so that the confusion in this field could be addressed.

With these questions answered, an insight into the potential benefits behind the pharmacological alteration of energy metabolism in the hypertrophied rat heart may be realized.

Table 1-1: Effects of L-carnitine deficiencies on myocardial function and energy metabolism.[1]

Carnitine Deficient State	Effect on Myocardial Function	Effect on Energy Metabolism	Reference
Primary and secondary carnitine deficiencies	Depressed myocardial function	Presumed depression of fatty acid oxidation	Didonato et al., 1992
Experimental Depletion:			
Na^+ pivalate	Depressed myocardial function with extended treatment	Depressed fatty acid oxidation with enhanced glucose oxidation rates	Broderick et al., 1995a
D-carnitine supplementation	Significant impairment of myocardial function	Probable depression of fatty acid oxidation	Paulson et al., 1984
Myocardial Hypertrophy	Depressed myocardial function	Fatty acid oxidation depressed Glycolysis enhanced Carbohydrate oxidation depressed in presence of high fat	Allard et al., 1994c Schönekess et al., 1995a El Alaoui-Talibi et al., 1992
Diabetes	Depressed myocardial function	Primary source of ATP from fatty acid oxidation Depressed carbohydrate metabolism	Paulson et al., 1984 Paulson et al., 1992
Reperfusion following ischemia	Depressed myocardial function	Fatty acid oxidation increased Glucose oxidation depressed	Lopaschuk et al., 1990 Renstrom et al., 1989 Görge et al., 1991 Liedtke et al., 1988a

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[1] Taken from Schönekess BO and Lopaschuk GD. The Effects of Carnitine on Myocardial Carbohydrate Metabolism. In: The Carnitine System and the Heart, de Jong, J. W., Ferrari, R. (eds.), Kluwar Academic Publishers, Dordrecht/Boston/London. (1995).

Table 1-2: Acute L-carnitine or propionyl L-carnitine loading of aerobically perfused normal, diabetic or hypertrophied rat hearts: the major effects on energy substrate preference and ATP contribution.[2]

Perfusion Condition	Energy Preference and Major ATP Source	Reference
Normal Rat Heart:		
- L-carnitine	Fatty acid oxidation primary source of ATP Carbohydrates supply 5-15% of ATP	Broderick et al., 1992
+ L-carnitine (10 mM for 90 min)	Increased carbohydrate contribution to ATP production mostly via an increased PDH flux (glucose oxidation) Decreased contribution of fatty acid oxidation to ATP production	
+ L-propionyl carnitine (1 mM for 60 min)	Increased carbohydrate contribution to ATP production via and increased PDH flux (glucose and lactate oxidation) Decreased contribution of fatty acid oxidation to ATP production	Schönekess et al., 1995a
Diabetic Rat Heart		
- L-carnitine	Almost all ATP from fatty acid oxidation. Carbohydrate metabolism almost non-existent	Broderick et al., 1995b
+ L-carnitine (10 mM for 60 min)	Dramatic increase in glucose contribution to ATP production (both glycolysis and glucose oxidation increase)	
Hypertrophied Rat Heart		
- L-propionyl carnitine	Majority of ATP from fatty acid oxidation Increased contribution of glycolysis and decreased contribution of glucose and lactate oxidation to ATP production	Schönekess et al., 1995a
+ L-propionyl carnitine (1 mM for 60 min)	No change in fatty acid oxidation Increased contribution of glucose and lactate oxidation to ATP production via an increased flux through PDH	

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[2] Taken from Schönekess BO and Lopaschuk GD. The Effects of Carnitine on Myocardial Carbohydrate Metabolism. In: The Carnitine System and the Heart, de Jong, J. W., Ferrari, R. (eds.), Kluwar Academic Publishers, Dordrecht/Boston/London. (1995).

Table 1-3: Effects of chronic L-carnitine or propionyl L-carnitine treatment on myocardial function.[3]

Treatment Regimen	Results on Myocardial Function	Reference
L-carnitine treatment (oral 4 g·d^{-1} for 1 yr) of humans with recent myocardial infarction	Increased heart rate, systolic and diastolic pressure, lower mortality, decreased anginal attacks, and rhythm disorders	Davini et al., 1992
L-carnitine treatment of humans with cardiopathies	Improved physical performance, decreased anginal attack rate and therapeutic use of nitrates	Fernandez and Proto, 1992
L-carnitine treatment (oral 1.8 g·d^{-1} for 4-8 wk) of human patients with stable angina pectoris	Improved exercise tolerance	Sotobat et al., 1989
L-propionyl carnitine treatment (oral 60 mg·kg^{-1} for 8 wk) in aortic-banded rats	Improved cardiac work (*in vitro*, isolated working hearts)	Schönekess et al., 1995b
L-propionyl carnitine treatment (ia 50 mg·kg^{-1} for 4 d) of aortic-banded rats	Improved cardiac function (*in vivo)* Lowered left ventricular end-diastolic pressure and increased relaxation rate (*in vitro*)	Motterlini et al., 1992 Yang et al., 1992
L-propionyl carnitine treatment (ip 100 mg·kg^{-1} for 8 wk) of diabetic rats	Improved post-ischemic contractile performance	Paulson et al., 1992

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[3] Taken from Schönekess BO and Lopaschuk GD. The Effects of Carnitine on Myocardial Carbohydrate Metabolism. In: The Carnitine System and the Heart, de Jong, J. W., Ferrari, R. (eds.), Kluwar Academic Publishers, Dordrecht/Boston/London. (1995).

Figure 1-1: Key sites at which L-carnitine potentially modulates fatty acid and carbohydrate oxidation in the heart.[4]

(1, lactate dehydrogenase complex; 2, acetyl CoA carboxylase; 3, acyl CoA synthetase; 4, carnitine palmitoyltransferase I; 5, carnitine acetyltransferase; 6, pyruvate dehydrogenase complex; 7, carnitine-acetylcarnitine translocase; 8, carnitine-acylcarnitine translocase; 9, carnitine palmitoyltransferase II; 10, β-oxidation)

-Carnitine ensures an adequate supply of intramitochondrial acetyl CoA from fatty acids at the level of 4, 8, and 9. In situations of adequate acetyl CoA supply for β-oxidation, carnitine can also act to lower the intramitochondrial acetyl CoA/CoA ratio at the level of 5 and 7. This will increase pyruvate dehydrogenase complex activity, and therefore glucose oxidation.

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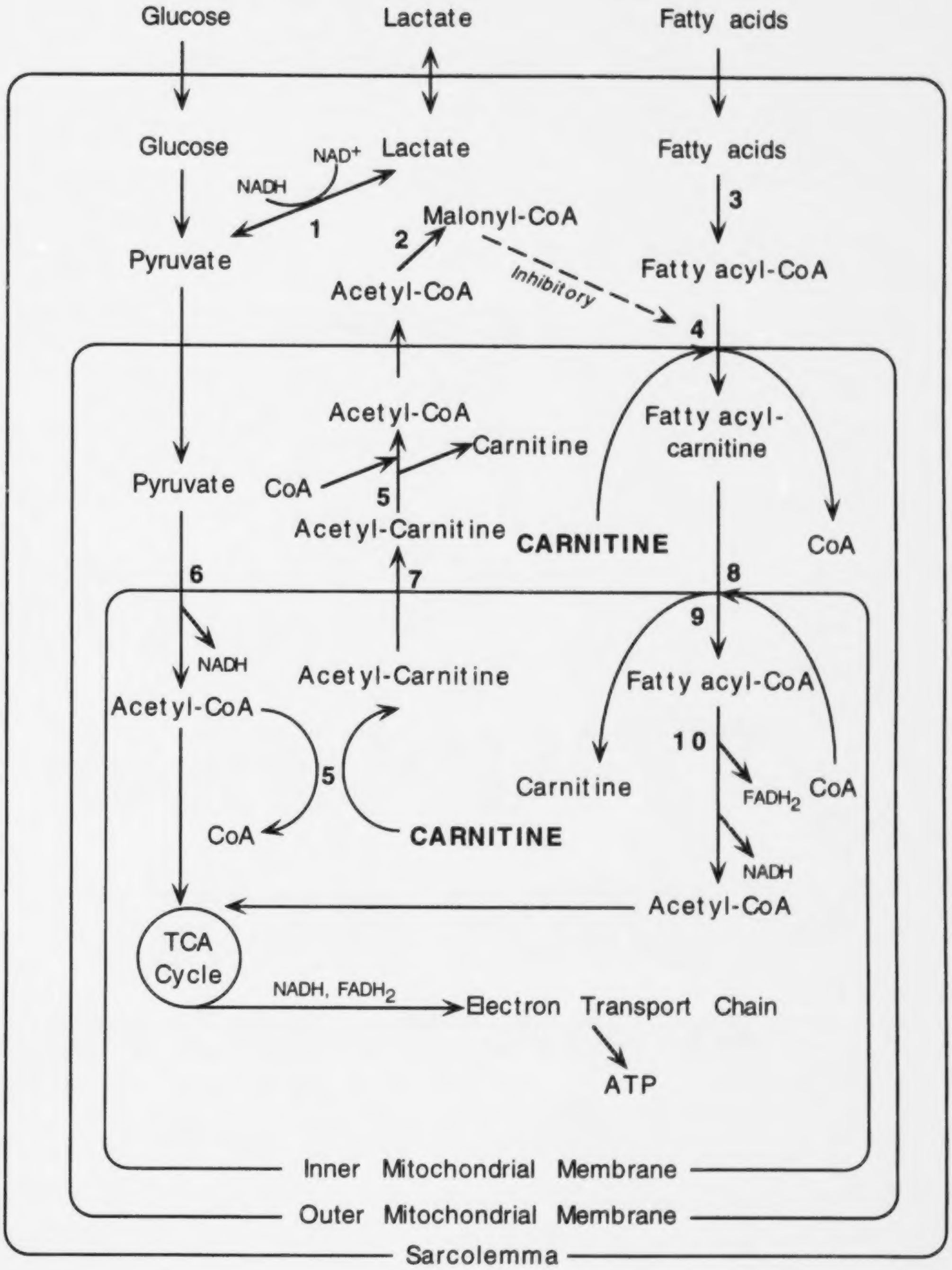
[4] Taken from Schönekess BO, and Lopaschuk GD. The effects of Carnitine on Myocardial Carbohydrate Metabolism. In: The Carnitine System and the Heart, de Jong JW, and Ferrari R (eds), Kluwar Academic Publishers, Dordrecht/Boston/London. (1995).


Glucose
Lactate
Fatty acids
Glucose
NAD+
Lactate
Fatty acids
NADH
3
1
Malonyl-CoA
2
Pyruvate
Fatty acyl-CoA
Acetyl-CoA
Inhibitory
4
Acetyl-CoA
Fatty acyl-carnitine
Carnitine
Pyruvate
CoA
5
Acetyl-Carnitine
CARNITINE
CoA
6
7
8
9
NADH
Acetyl-Carnitine
Fatty acyl-CoA
Acetyl-CoA
10
5
Carnitine
FADH2
CoA
CoA
CARNITINE
NADH
Acetyl-CoA
TCA Cycle
NADH, FADH2
Electron Transport Chain
ATP
Inner Mitochondrial Membrane
Outer Mitochondrial Membrane
Sarcolemma

Chapter II

General Experimental Methods

A) Materials

D-[5-^{3}H]glucose, D-[U-^{14}C]glucose, [U-^{14}C]lactate and [9,10-^{3}H(N)]palmitate were purchased from Du Pont-New England Nuclear. Bovine serum albumin (fraction V) was obtained from Boehringer Mannheim, Germany. Hyamine hydroxide (methylbenzethonium; 1 M in methanol solution) was obtained from Sigma Chemical Company (St. Louis, Missouri). Dowex 1X4 anion exchange resin (200-400 mesh chloride form), Dowex AG50 WX8 (200-400 mesh, hydrogen form), and Dowex AG1X8 (200-400 mesh, acetate form), were all obtained from Bio-Rad (Richmond, Virginia). Silicic acid (325 mesh) was obtained from BDH Chemicals LTD (Poole, England) ACS counting scintillant was purchased from Amersham Canada Ltd (Oakville, Ontario). Ecolite counting scintillant was obtained from ICN Biomedicals Canada Ltd (Mississauga, Ontario). PLC (lot #920068) was obtained from Prassis Sigma Tau Research Institute (Milano, Italy). All other chemicals were reagent grade.

B) Animal model for the production of myocardial hypertrophy

All animal use, housing, surgical manipulations, and euthanization have been approved by the Health Sciences Animal Welfare Committee at the University of Alberta, and conform to the standards set by the Canadian Council of Animal Care.

Male Wistar-Kyoto (**CHAPTERS III,V,VI**) or Sprague-Dawley (**CHAPTERS IV,VII**) rats, age 3 weeks (50-70 g), were anaesthetized with an intraperitoneal injection of methohexital sodium (50 $mg \cdot kg^{-1}$). The suprarenal abdominal aorta was exposed through a lateral incision in the abdominal wall, isolated, and restricted with a hemoclip (0.40 mm diameter). Control animals had the aorta isolated but did not have the hemoclip applied. The abdominal incision was then closed and the animals allowed to recover. Food and water were administered *ad libitum*. All hypertrophied heart experiments were performed on hearts obtained from animals eight weeks after surgery. This banding technique relies upon the animals' natural growth to produce a gradually increasing degree of aortic constriction.

No post-operative antibiotics or analgesics were given to these animals. Post-operative survival rates were greater than 95%.

C) Isolated heart preparation

Hearts from pentobarbital-anaesthetized control or aortic-banded rats were excised, placed in ice-cold buffer, and the aorta was cannulated within 60 sec of removal from the animal (Lopaschuk and Spafford, 1989; Lopaschuk et al., 1990). Hearts were initially perfused via a retrograde perfusion of the aorta in the Langendorff mode at 60 mm Hg, with oxygenated Krebs-Henseleit buffer, pH 7.4. The initial Langendorff perfusion buffer contained either, 5.5 or 11 mM glucose and 1.25 or 2.50 mM Ca^{2+}

depending on the perfusion protocols used for the individual experiments. The isolated working heart buffer additionally contained 0.5 mM lactate (a range of lactate concentrations from 0.0-8.0 mM was used in **CHAPTER IV**), 0.4 or 1.2 mM palmitate prebound to 3% bovine serum albumin, and 100 $\mu U \cdot ml^{-1}$ insulin, again depending on the perfusion protocols used for the individual experiments (specific concentrations of carbon substrate and free Ca^{2+} are outlined in the appropriate *Methods* sections of each chapter). Perfusion buffer was recirculated and had a volume was 100 ml, except for the experiments done in **CHAPTER IV**, where the perfusion buffer volume was increased to 150 ml. A 95% O_2 and 5% CO_2 compressed air mixture was bubbled through the Langendorff buffer to maintain oxygenation, and pH. This same gas mixture was also used for oxygenation of the isolated working heart perfusate. Temperature for all perfusion experiments was maintained at 37°C. It was during the initial retrograde aortic perfusion, that hearts were trimmed of excess tissue and the left atrium was cannulated to allow for filling of the left atria with perfusion buffer. This allowed for a working heart model where perfusion buffer would enter the left atria via the canula, be ejected into the left ventricle, and subsequent ejection of the perfusion buffer from the left ventricle into the aortic cannula. Unless otherwise stated, preload pressure (left atrial pressure) for the working heart mode was set at 11.5 mm Hg. Afterload pressure (aortic pressure) was set at 80 mm Hg.

Heart rate (HR) and peak systolic pressure (PSP) were recorded using a Gould RS-3600 physiograph with a Spectramed P 23XL pressure transducer in the aortic afterload line. Developed pressure (dP) was calculated as PSP-diastolic pressure.

Cardiac output (CO) and aortic output (AO) were also measured using Transonic ultrasonic flow probes present in the left atrial inflow line and the aortic outflow line. Coronary flow (CF) was calculated as the difference between cardiac output and aortic output. When O_2 consumption was measured, the pulmonary artery was also cannulated (Lopaschuk et al., 1988). The effluent from the pulmonary artery was directed past a YSI oxygen electrode. The difference in oxygen content of the preload perfusate which enters the left atria, and the oxygen content of the perfusate leaving the pulmonary artery was used to calculate the amount of oxygen (MVO_2) used by the heart. These calculations are dependant on the coronary flow of the individual hearts and the percent saturation of the perfusate with oxygen.

D) Measurement of myocardial glycolysis

To measure glycolytic rates in the isolated working heart preparation, we incorporated [5-^{3}H]glucose into the perfusion buffer. Rates of glycolysis were measured by quantitative determination of the amount of ^3H_2O liberated from the labelled [5-^{3}H] glucose at the enolase step of glycolysis (Saddik and Lopaschuk, 1991). ^3H_2O was separated from [^{3}H]glucose and [^{14}C]glucose (used to measure glucose oxidation, see following section) using chromatography columns containing Dowex 1X4 anion exchange resin (200-400 mesh) suspended in 0.4 M potassium tetraborate. The Dowex resin in the columns (resin volume of 0.4 ml) was extensively washed with H_2O prior to use. A 0.2 ml volume of perfusate was added to the column and eluted into scintillation vials with

0.8 ml of H_2O. Following addition of Ecolite scintillant, the samples were subjected to standard double isotope counting procedures to detect ^3H_2O and residual [^3H/^{14}C]glucose. The Dowex columns were found to retain 98-99.6% of the total [^3H]-glucose and [^{14}C]-glucose present in the perfusate. The ^3H_2O was corrected for the small amount of [^3H]glucose that passed through the column. This could be accomplished since an equal amount of [^{14}C]glucose also passed through the column and could be used as an internal standard for the degree of [^3H]glucose contamination in the ^3H_2O sample. Correction was also made for the degree of spillover from [^{14}C] into the [^3H] counting window by measuring this degree of spillover using standards containing only [^{14}C]glucose. Glycolytic rates are expressed as nmol of glucose metabolized$\cdot$min$^{-1}\cdot$g dry wt^{-1}.

E) Measurement of myocardial glucose oxidation

Rates of glucose oxidation were measured by quantitatively collecting $^{14}CO_2$ produced by the heart from the TCA cycle and PDH from [U-^{14}C] glucose in the buffer, as described previously (Saddik and Lopaschuk, 1991). $^{14}CO_2$ produced as a gas was collected by bubbling gas from the closed perfusion system through a hyamine hydroxide $^{14}CO_2$ trap (25 or 40 ml of hyamine hydroxide in the trap depending on perfusion time). This allowed for the quantitative collection of all $^{14}CO_2$ released as a gas. Perfusate samples (4 ml) were collected and stored under mineral oil to prevent the liberation of $^{14}CO_2$ (present as $H^{14}CO_3^-$) by the equilibration of atmospheric CO_2. Two, 1 ml samples of the perfusate were subsequently injected into closed metabolic reaction flasks containing 1 ml of 9 N H_2SO_4, and gently shaken for 1 hr. This procedure released $^{14}CO_2$ from the perfusion buffer that was present in the form of $H^{14}CO_3^-$. The $^{14}CO_2$ was trapped in centre wells fitted with filter paper saturated with 0.4 ml of hyamine hydroxide, or trapped in scintillation vials containing filter paper and 0.3 ml of hyamine hydroxide. The centre wells (or scintillation vials) were removed and ACS counting scintillant was added. Standard β-scintillation counting procedures were used to detect $^{14}CO_2$. Rates of glucose oxidation are expressed as nmol of glucose metabolized·min^{-1}·g dry wt^{-1}.

F) Measurement of myocardial lactate oxidation

Rates of lactate oxidation were measured in the same hearts as rates of palmitate oxidation. This is done by incorporating [U-^{14}C]lactate and [9,10-^{3}H(N)]palmitate in the perfusion buffer. When lactate and palmitate oxidation were measured, they were done in a parallel series of hearts following the same protocol as hearts used to measure glycolysis and glucose oxidation. $^{14}CO_2$ production occurs as lactate (following oxidation to pyruvate by LDH) passes through PDH and subsequently into the TCA cycle. A similar procedure is used to measure the $^{14}CO_2$ produced as a gas and in the perfusate as $H^{14}CO_3^-$, as described above for the measurement of glucose oxidation. Rates of lactate oxidation are expressed as nmol lactate metabolized·min^{-1}·g dry wt^{-1}.

G) *Measurement of myocardial palmitate oxidation*

Rates of palmitate oxidation were measured by quantitative determination of the rate of ^3H_2O production from [9,10-^{3}H]palmitate, as described previously (Saddik and Lopaschuk, 1991). This method entails the extraction of a 0.5 ml sample of perfusion buffer with 1.88 ml of a 1:2 v/v mixture of chloroform:methanol, followed by the addition of 0.625 ml $CHCl_3$, and 0.625 ml of a 2 M KCl:HCl solution. After separation into aqueous and organic chloroform phases, the aqueous phase is taken and initially extracted with 1.0 ml $CHCl_3$, 1.0 ml of MeOH, and 0.9 ml of 2 M KCl:HCl. This method results in a greater than 99.7% extraction and separation of ^3H_2O from [9,10-^{3}H]palmitate (Saddik and Lopaschuk, 1991). Duplicate samples of the aqueous phase (0.5 ml) were taken and counted to determine the content of ^3H_2O and [^{14}C]lactate after addition of Ecolite counting scintillant. Spillover of [^{14}C]lactate into the aqueous phase of the extract occurs and is taken into account by using standard dual counting procedures in a ß-scintillation counter.

H) *Calculation of ATP production from steady state rates of glycolysis, glucose oxidation, lactate oxidation, and palmitate oxidation*

Steady state rates of ATP production from substrate metabolism was calculated as follows: 1 mol of glucose passing through glycolysis forms 2 mol of ATP; 1 mol of glucose passing through glucose oxidation forms 36 mol of ATP; 1 mol of lactate passing through lactate oxidation forms 18 mol of ATP; and 1 mol of palmitate passing through palmitate oxidation forms 129 mol ATP. This method of calculating ATP production assumes that 100% of the NADH and $FADH_2$ is used in the production of the proton gradient across the inner mitochondrial membrane, and that there is no loss of this gradient prior to ATP synthesis by ATP synthase. A recent publication by Brand et al., (1994) suggests that there may be a considerable loss of the proton motive force across the inner mitochondrial membrane in isolated hepatocytes and skeletal muscle. The substantial rate of proton leak can therefore affect the efficiency of oxidative phosphorylation and the production of ATP. Brand et al., (1994) further suggest that proton leak and the resulting respiration driven proton pumping may be the single most important consumer of free energy in animals. Although there is no evidence as to the effects of proton leak in myocardial mitochondria, the substantial ATP demand of the working heart may actually decrease the importance of this phenomenon.

I) Myocardial Metabolites

i) Dry to wet ratios

After each perfusion, the ventricles of the hearts were frozen with Wollenberger clamps cooled to the temperature of liquid nitrogen. The frozen ventricular tissue was weighed and powdered in a mortar and pestle cooled to the temperature of liquid nitrogen. A portion of the tissue was used to determine the dry-to-wet-weight ratio. The total dry weight of the heart was determined from the dry-to-wet ratio and the frozen ventricular weight and the weight of the dried atrial tissue.

ii) High energy phosphates

Neutralized perchloric acid extracts of frozen ventricular tissue were used to measure ATP, creatine, and creatine-phosphate by high pressure liquid chromatograpy (HPLC) analysis (Stocchi et al., 1987; Ally and Park., 1992). Myocardial tissue (100 mg) was extracted with 1 ml 6% PCA/0.5 mM ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) solution, followed by centrifugation and neutralization with K_2CO_3 (5 M). The supernatant was taken and analyzed by HPLC[5]. This method entailed the use of a LC18-T (5 μm particle size, 250 mm x 4.6 mm I.D., Supelco Canada, Oakville Ontario) chromatography column. The mobile phase consisted of two buffers. Buffer A was 35 mM KH_2PO_4, 6 mM tetrabutylammonium hydrogensulfate, pH 6.0, 125 μM EDTA. Buffer B was a mixture of Buffer A and HPLC-grade acetonitrile (1:1 v/v). Buffers were filtered through a 0.2 μm membrane filter, and helium-gassed. After 10 min of isocratic elution with a 98% Buffer A and 2% Buffer B, the gradient was changed to 45% Buffer A and 55% Buffer B. This took place over 10 min. After reaching this gradient, a further 5 min run took place, at a flow rate of 1.0 $ml \cdot min^{-1}$. The column was re-equilibrated after each run to 98% Buffer A and 2% Buffer B.

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[5] HPLC analysis was performed by Mr. K. Strynadka.

iii) Carnitine esters and total carnitine

Extraction of free, short-chain and long-chain acyl carnitines was performed as previously described (Broderick et al., 1992). This involved extraction of free and short chain carnitine esters from long-chain carnitine esters with 6% PCA (initially 400 mg tissue and 1.5 ml 6% PCA). Alkaline hydrolysis of a 500 μl aliquot of the initial supernatant (which contains both free carnitine and short-chain carnitine esters) from the perchloric acid extract with KOH (pH 11.5, 55 °C, for 15 min) yielded total acid soluble carnitine in the free, unesterified form. The long-chain carnitine esters were extracted from the pellet of the PCA extract by alkaline hydrolysis (KOH, pH 13, 70 °C, for 60 min). Levels of free carnitine from the initial neutralized extract, and from the hydrolytic fractions were measured using a radiometric assay (McGarry and Foster, 1976).

Total myocardial carnitine content was determined by exposing a small quantity of frozen tissue (50 mg) to a period of alkaline hydrolysis (1 ml KOH, pH 13, 70 °C for 60 min). This results in the hydrolysis of short- and long-chain carnitine esters and the production of free carnitine. A radiometric assay was used to determine the total free carnitine content of the extract (McGarry and Foster, 1976).

iv) Lactate content

Neutralized PCA extracts of frozen ventricular tissue used to measure high energy phosphates (see Section iii) was also used to measure tissue lactate. The tissue was extracted with a 6% PCA/0.5 mM EGTA solution, followed by centrifugation and neutralization with K_2CO_3 (5 M). The resultant supernatant (containing tissue lactate) was measured using a spectrophotometric assay involving LDH (Bergmeyer and Grassl, 1983).

v) Glycogen content

Glycogen content was measured by alkaline extraction (500 μl 10 M KOH, pH 13, 100 °C, 60 min) of tissue (250 mg), ethanol precipitation (2 ml, 95% ethanol, overnight in -20 °C freezer), and acidic hydrolysis (1 ml 10 N H_2SO_4, pH 1, 100 °C, 180 min) of glycogen to yield free glucose. A spectrophotometric assay was then used to determine the glucose content (Bergmeyer and Grassl, 1983).

vi) Triacylglycerol content

Myocardial triacylglycerol was measured by a modified method of Saddik and Lopaschuk 1991, Bowyer and King 1977, and Lopaschuk et al. 1986. This involved the homogenization of 100 mg tissue in 4 ml of ice-cold chloroform/methanol (2:1 v/v). Following the addition of 0.6 ml of methanol, the mix was centrifuged and the supernatant was collected. Evaporation of the solvent was carried out at 50 °C under $N_{2(g)}$. The lipids were redissolved in 2.5 ml of chloroform, and separated on a silicic acid column (2.5 ml, of 28.5 g silicic acid suspended in 100 ml chloroform). Elution and separation of the neutral lipids (triacylglycerol) from phospholipids was achieved by the addition of 4 ml chloroform. The eluent was dried down at 50 °C under $N_{2(g)}$. Assay for total myocardial triglycerides was carried out using an enzymatic reaction involving lipoprotein lipase to form glycerol, and the subsequent formation of a red colour compound (Wako Triglyceride G kit, Japan)

J) Measurement of lactate release

The method adapted for the separation of lactate from the perfusion buffer was adapted from the method used by the laboratory of Dr. J. Wisneski[6]. Two sets of chromatography columns were prepared for the separation of [U-^{14}C]lactate from [U-^{14}C]glucose. The first set of columns were filled with 3 ml of a 10% w/v slurry of activated and neutralized AG50 WX8 Dowex column (200-400 mesh, hydrogen form). The second set of columns were filled with 5 ml of a 13% w/v slurry of washed AG1X8 Dowex (200-400 mesh, acetate form). The columns were drained and the first set (AG50) were placed on top of the second set (AG1X8). 200 μl of perfusion buffer containing [U-^{14}C]lactate and [U-^{14}C]glucose was added to the top of the columns. Afterwards two, 750 μl aliquot's of ddH$_2$O was added and allowed to drain from both columns. After the first set of columns was drained, they were removed and discarded. To the second set of columns, a 500 μl aliquot of ddH$_2$O was added followed by 500 μl of 0.5 M sodium acetate. After the columns had run dry, the eluent from these columns discarded. Placing the columns over scintillation vials, two, 1000 μl aliquot's of 1.0 M sodium acetate were added. After the columns had drained, they were discarded and 10 ml of Ecolite scintillation fluid was added to the scintillation vials containing the eluent. This extraction procedure resulted in greater than 97% recovery of [U-^{14}C]lactate, with less than 0.1% contamination by [U-^{14}C]glucose.

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[6] As relayed to our laboratory by Dr. W. C. Stanley.

K) Statistical Analysis

Data are expressed as the mean ± standard error of the mean (SEM). Comparison between groups was performed using the unpaired Students 't'-test. Multiple comparisons (and comparisons within groups) were made by ordinary Anova, using a Student Newman-Keull's post-test. Significance was set at $p \leq 0.05$.

The calculation for SEM of data expressed in stack graphs, i.e., total ATP production, was calculated as follows:

z = x + y + ...

SEM (z) = SQRT (SEM (x)^2 + SEM (y)^2 + ...)

where: z is the result of combined data x, y, and ...

SEM (z) is the SEM of z (the result of the combined data)

SEM (x) is the SEM of x (a component of the combined data)

SEM (y) is the SEM of y (a component of the combined data)

SQRT is the square root

This formulation was found in a Physics 241/243 laboratory manual (University of Alberta). Original reference Taylor 1982.

CHAPTER III

Contribution of oxidative metabolism and glycolysis to ATP production in hypertrophied hearts during high and low work states[7]

A) Introduction

Considerable controversy exists as to whether rates of glycolysis or the glycolytic capacity is enhanced or decreased in hypertrophied hearts. Activities of enzymes involved in glycolysis measured in hypertrophied myocardium are increased compared to non-hypertrophied control hearts (Taegtmeyer and Overturf, 1988). Also, the isozymes of LDH (Bishop and Aultschuld, 1971) and creatine kinase (Ingwall, 1984) are shifted to more fetal anaerobic forms. Lactate production by the hypertrophied myocardium is increased during hypoxia (Bishop and Aultschuld, 1971; Anderson et al., 1990a) and ischemia (Bladergroen et al., 1990) relative to normal hearts. In contrast, Cunningham *et al.*, (1990) have suggested that lactate production is decreased in the hypoxic hypertrophied heart. Although there have been indirect measures of glycolysis in the hypertrophied heart, no direct measurements of glycolysis or glucose oxidation have previously been made prior to this study.

It is well established that L-carnitine levels are decreased in the hypertrophied myocardium (Reibel et al., 1983; El Alaoui-Talibi et al., 1992; Motterlini et al., 1992;

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[7] A version of this chapter has been published. Allard MF, Schönekess BO, Henning SL, English DR, and Lopaschuk GD. 1994. Am J Physiol 267: H742-750.

Yang et al., 1992; Cheikh et al., 1994; Michelletti et al., 1994ab). L-carnitine is an essential co-factor required for the translocation of activated long-chain fatty acids into the inner mitochondrial matrix. A limitation in the translocation of fatty acids into the mitochondria due to decreased L-carnitine levels has been suggested to lead to decreased ATP production from fatty acid oxidation, and thereby myocardial dysfunction (Wittels and Spann, 1968; Whitmer, 1986; El Alaoui-Talibi et al., 1992; Cheikh et al., 1994). Although fatty acid oxidation has been shown to be decreased in volume-overloaded isolated perfused hypertrophied hearts (El Alaoui-Talibi et al., 1992), the contribution of exogenous fatty acids to ATP production has not been characterized under perfusion conditions using physiological free fatty acid concentrations (0.4 mM palmitate).

Measurements of oxidative metabolism and glycolysis were therefore carried out to determine the relative contribution of glycolysis and mitochondrial oxidation of glucose, lactate, and palmitate oxidation to ATP production in isolated working hypertrophied rat hearts. Since workload is an important determinant of glycolysis and oxidative metabolism (Neely and Morgan, 1974; Neely et al., 1976), energy substrate metabolism was measured in hearts perfused at both low and high workloads.

B) Methods

i) Hypertrophy model

Myocardial hypertrophy was produced in male Wistar-Kyoto rats as described in

ii) Specific perfusion protocol

Following cannulation of the aorta and left atria, control and hypertrophied hearts were perfused in the working heart mode with a perfusion buffer containing 11 mM glucose, 0.5 mM lactate, 0.4 mM palmitate bound to 3% bovine serum albumin, 1.25 mM Ca^{2+} and 100 $\mu U \cdot ml^{-1}$ insulin. Hearts were perfused for 30 min at an afterload pressure of 60 mm Hg (low work), followed by an increase in afterload to 120 mm Hg (high work) to produce an increase in myocardial work for an additional 30 min. The order in which the workload was imposed on the hearts, i.e., low work and then high work, remained constant and was not randomized.

Glycolysis, glucose oxidation, lactate oxidation, and palmitate oxidation were measured in hearts perfused at both workloads (low and high work) as described in **CHAPTER II**. Buffer samples were taken at 10 min intervals throughout the perfusion. At the end of the 60 min perfusion hearts were quick frozen by clamping with Wollenberger clamps cooled to the temperature of liquid nitrogen.

B) Results

i) Heart weight and body weight of experimental animals

The heart weight and body weight data are summarized in Table 3-1. Eight weeks following surgery the body weight of the aortic-banded rats tended to be less than that of the control rats, but this difference was not statistically significant. Total wet heart weight was 38% greater in the aortic-banded rats than the control rats. When total wet heart weight was normalized to the corresponding body weight, the heart weight/body weight ratio was 45% greater in the aortic-banded rats compared to control rats.

ii) Mechanical function in control and hypertrophied hearts

Mechanical function in isolated working hearts from control and aortic-banded rats is shown in Table 3-2. Mechanical function at both a low workload (60 mm Hg aortic afterload) and a high workload (120 mm Hg afterload) was measured. Spontaneous HR at the low work was not different between control and hypertrophied hearts and an increasing workload did not significantly alter HR in either experimental group. At the 11.5 mm Hg left atrial preload used in these experiments the hypertrophied hearts had a significantly lower PSP at low work compared to the control hearts. However, at high work these differences in PSP did not occur. Left ventricular systolic function, expressed as the product of HR and PSP, significantly increased in both control and hypertrophied hearts following the transition to high work. There were no differences in HR·PSP between the control and the hypertrophied hearts at either the low or the high workloads.

In addition to HR and PSP measurements, cardiac output, aortic output, and coronary flow were also monitored in these hearts. At low work, cardiac output was significantly depressed in the hypertrophied hearts. The main component of cardiac output in both groups was aortic flow. In both experimental groups cardiac output tended to decrease when aortic afterload was increased to 120 mm Hg. However, the majority of cardiac output in these hearts now became coronary flow, with a marked decrease in aortic flow. At this high workload, cardiac output remained depressed in the hypertrophied hearts. There were no differences in rates of absolute coronary flow between control and hypertrophied hearts during either the low or high work periods. However, when coronary flow was normalized per g dry weight, the hypertrophied heart had a significantly lower coronary flow rate at both low and high work.

iii) Myocardial rates of glycolysis and glucose oxidation at low and high work

Steady state rates of glycolysis and glucose oxidation in control and hypertrophied hearts are shown in Figure 3-1. Confirming previous results (Saddik and Lopaschuk, 1991), glycolytic rates are substantially higher than glucose oxidation rates in control hearts when hearts are perfused in the presence of physiologically relevant concentrations of free fatty acids (0.4 mM palmitate). This is because fatty acids are more potent inhibitors of glucose oxidation than of glycolysis (Saddik and Lopaschuk, 1991). During low work, there were no significant differences in the rate of oxidation of glucose between control and hypertrophied hearts. Glycolytic rates, however, were significantly elevated in the hypertrophied hearts.

Under conditions of high work, a greater than 3-fold increase in glucose oxidation rates were seen in both control and hypertrophied hearts (Figure 3-1), with no significant differences between the two groups. During high work, the control hearts showed a significant increase in the rates of glycolysis compared to rates at low work. Glycolytic rates in the hypertrophied hearts remained unchanged in the high work state, resulting in glycolytic rates that were similar in control and hypertrophied rat hearts at high work.

iv) Myocardial rates of lactate oxidation at low and high work

As shown in Figure 3-2, there were no significant differences in the rate of lactate oxidation between control and hypertrophied hearts during the low work period. Similar to what was seen with glucose oxidation, high work resulted in a significant increase in lactate oxidation in both control and hypertrophied hearts. There were no significant differences between control and hypertrophied hearts.

v) Myocardial rates of palmitate oxidation at low and high work

During low work, steady state palmitate oxidation rates were decreased in the hypertrophied hearts compared to control hearts (Figure 3-3). In both control and hypertrophied hearts rates of palmitate oxidation did not increase during the transition to high work. During the high work period there were no significant differences between control and hypertrophied hearts, although the hypertrophied hearts tended to have lower oxidative rates.

vi) Rates of glycolysis and oxidative metabolism normalized for mechanical function

Mechanical function is an important determinant of both glycolysis and oxidative metabolism (Neely and Morgan, 1974; Neely et al., 1976). To account for any work induced increase in the rates of steady state glycolysis or oxidative metabolism, rates were normalized for mechanical function. As shown in Table 3-3, the increased glycolytic rates in hypertrophied hearts seen at low work remained. The dramatic increase in glucose oxidation following the transition from low to high work also remained in both the control and hypertrophied hearts.

Lactate oxidation rates normalized for mechanical work are also shown in Table 3-3. The increase in lactate oxidation following the transition from low to high work persisted in these hearts. Again no significant differences between control and hypertrophied rat hearts were seen. At low work, the decreased rates of palmitate oxidation seen in hypertrophied hearts were still evident. Interestingly, at high work, palmitate oxidation rates normalized for mechanical function were significantly decreased in control hearts. This was due to the fact that absolute rates of palmitate oxidation did not change in control hearts during the increase in workload (Figure 3-3) despite the significant increase in mechanical function. Again at high work, no significant differences in palmitate oxidation rates were seen between control and hypertrophied hearts.

vii) Myocardial ATP production in control and hypertrophied hearts at low and high work

The relative contribution of the glycolytic and oxidative pathways to steady state myocardial ATP production can be calculated by multiplying the amount of ATP produced per mol of substrate metabolized (described in **CHAPTER II**). Steady state rates of ATP production in control and hypertrophied hearts are shown in Figure 3-4. At low work (60 mm Hg afterload), the majority of ATP production was derived from the oxidation of palmitate. In hypertrophied hearts, overall ATP production from exogenous sources was decreased, with a greater contribution of glycolysis to ATP production and a lesser contribution of fatty acid oxidation.

At high work an increase in ATP production was seen in both control and hypertrophied hearts. Interestingly, almost all of this increase was accounted for by an increase in carbohydrate use. In both control and hypertrophied hearts the extra ATP necessary to sustain contractile function was primarily derived from an increase in glucose oxidation and to a lesser extent from an increase in lactate oxidation and glycolysis.

Determination of the percent contribution of glycolysis and oxidative metabolism to ATP production in control and hypertrophied hearts in which ATP production was normalized for differences in mechanical function was determined. Figure 3-5 shows the relative percent contribution of glycolysis, glucose oxidation, lactate oxidation, and palmitate oxidation to ATP production at each workload. At low work, the main difference between control and hypertrophied hearts was that the decreased contribution of fatty acid oxidation to ATP production in hypertrophied hearts was accompanied by an increase in the contribution of glycolysis to ATP production. The other interesting point is that at high work, the contribution of carbohydrate (glucose and lactate) oxidation as a source of ATP increased in both control and hypertrophied hearts, and actually became the major source of ATP production in hypertrophied hearts.

viii) Myocardial ATP content at the end of high work

To ensure that the hypertrophied hearts were not in an energy deficient state at the end of the high work period, hearts were frozen and the myocardial level of ATP measured. ATP levels in control and hypertrophied hearts were 28.5 ± 2.2 (n=9) and 35.5 ± 4.2 μmol·g dry wt^{-1} (n=9), respectively. This demonstrates that the measurements of ATP production rates in the hypertrophied hearts were not compromised by hearts being in an energy deficient state.

ix) Myocardial L-carnitine content in control and hypertrophied rat hearts

Previous studies have suggested that decreased L-carnitine levels observed in hypertrophied hearts may be responsible for alterations in myocardial metabolism. Therefore the levels of L-carnitine esters in hearts frozen following the perfusion protocol were measured (Table 3-4). In hypertrophied hearts, a decrease in total L-carnitine content was seen compared to control hearts, which was primarily the result of a decrease in free L-carnitine and to a lesser extent short chain L-carnitine.

D) Summary

From this data it can be concluded that the hypertrophied heart;

1) has an increased aerobic rate of glycolysis compared to normal heart,

2) has a depressed rate of fatty acid oxidation at low levels of work compared to normal heart,

3) increases rates of carbohydrate oxidation to provide the extra ATP required in response to increased external workloads,

4) has a lower myocardial blood flow when measured on a per g dry basis.

Table 3-1: Heart and body weight of control and aortic banded animals

	Dry Heart Weight (g)	Wet Heart Weight (g)	Body Weight (g)	Heart Weight/Body Weight Ratio ($\times 10^{-3}$)
Control (n=25)	0.31 ± 0.02	1.48 ± 0.04	292 ± 5	5.1 ± 0.2
Hypertrophied (n=32)	$0.39 \pm 0.03^*$	$2.04 \pm 0.07^*$	279 ± 6	$7.4 \pm 0.3^*$

* significantly different from control value ($p \leq 0.05$).

Table 3-2: Functional characteristics of control and hypertrophied hearts during low and high work

Parameter Measured	Low Work		High Work	
	Control (n=25)	Hypertrophied (n=32)	Control (n=25)	Hypertrophied (n=32)
Heart Rate (beats·min^{-1})	213 ± 13	218 ± 9	243 ± 11	246 ± 9
Peak Systolic Pressure (mm Hg)	94 ± 4	84 ± 2*	125 ± 4†	121 ± 34†
HR·PSP (beats·mm Hg·10^{-3})	19.7 ± 1.0	18.3 ± 0.7	29.2 ± 1.7†	26.5 ± 1.2†
Cardiac Output (ml·min^{-1})	34 ± 2	25 ± 2*	30 ± 2†	23 ± 2†
Aortic Output (ml·min^{-1})	20 ± 2	13 ± 2	5 ± 1†	2 ± 1†
Coronary Flow (ml·min^{-1})	15 ± 1	13 ± 2	25 ± 2†	21 ± 2†
Coronary Flow (ml·min^{-1}·g dry wt^{-1})	48 ± 1	33 ± 2*	81 ± 2†	54 ± 2*†

* significantly different from control value ($p \leq 0.05$).
† significantly different from comparable low work value ($p \leq 0.05$).

Table 3-3: Steady state values of glycolysis, glucose oxidation, lactate oxidation, and palmitate oxidation normalized for mechanical function

	Glycolysis	Glucose Oxidation	Lactate Oxidation	Palmitate Oxidation
	nmol·min^{-1}·g dry wt^{-1}·(HR·PSP·10^{3})$^{-1}$			
Control				
Low Work	113 ± 10	15 ± 2	10 ± 1	17 ± 3
High Work	114 ± 8 (n=9)	$27 \pm 3^{\dagger}$ (n=9)	$19 \pm 3^{\dagger}$ (n=12)	13 ± 3 (n=8)
Hypertrophied				
Low Work	$209 \pm 34^{*}$	10 ± 2	9 ± 1	$9 \pm 2^{*}$
High Work	146 ± 20 (n=11)	$22 \pm 3^{\dagger}$ (n=11)	$14 \pm 1^{\dagger}$ (n=12)	7 ± 2 (8)

[*] significantly different from control value ($p \leq 0.05$).

[†] significantly different from comparable low work value ($p \leq 0.05$).

Table 3-4: Myocardial carnitine content of control and hypertrophied hearts

	Free	Short chain	Long chain	Total
μmol·g dry wt^{-1}				
Control (n=7)	3458 ± 392	1998 ± 310	325 ± 71	5795 ± 684
Hypertrophied (n=7)	2424 ± 244*	1179 ± 260	496 ± 154	4099 ± 342*

* significantly different from control value ($p \leq 0.05$).

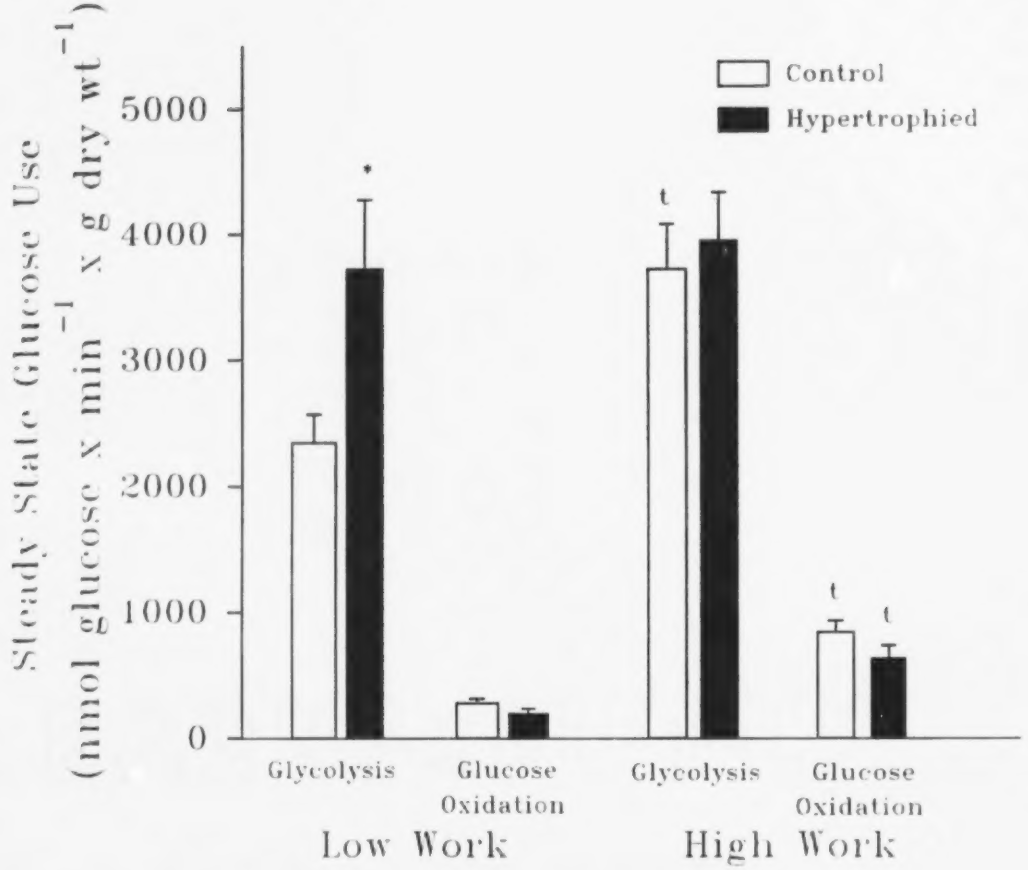

Figure 3-1: Steady state glucose use (glycolysis and glucose oxidation) in control and hypertrophied rat hearts subjected to low and high work states. Steady state rates of glycolysis and glucose oxidation were determined in hearts perfused with buffer containing 11 mM [5-^{3}H/U-^{14}C]glucose, 0.5 mM lactate, 0.4 mM palmitate and 100 μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of either 60 mm Hg (low work) or 120 mm Hg (high work). ^3H_2O and $^{14}CO_2$ production were measured as described in **CHAPTER II**. Values are the mean ± SEM n=7-8 hearts in each group. *significantly different from control hearts ($p \leq 0.05$). ^{t}significantly different from comparable hearts at low work ($p \leq 0.05$).

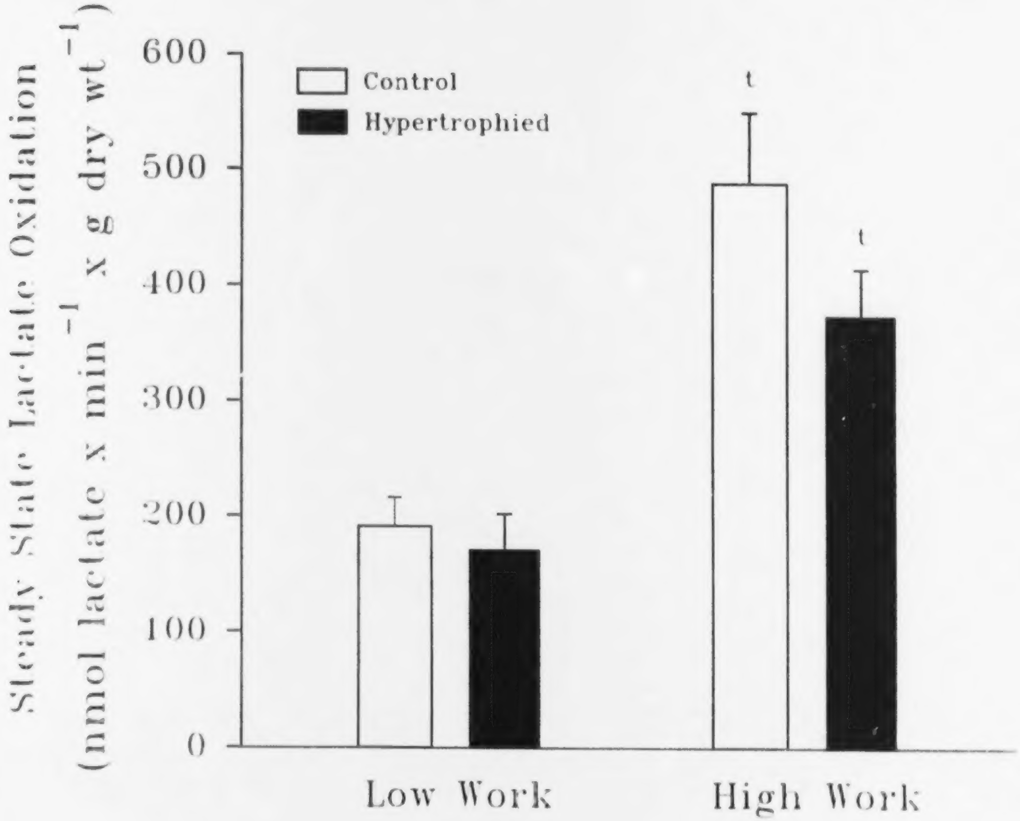

Figure 3-2: Steady state lactate oxidation in control and hypertrophied rat hearts subjected to low and high work states. Steady state rates of lactate oxidation were determined in hearts perfused with buffer containing 11 mM glucose, 0.5 mM [U-^{14}C]lactate, 0.4 mM palmitate, and 100 μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of either 60 mm Hg (low work) or 120 mm Hg (high work). $^{14}CO_2$ production from [U-^{14}C]lactate was measured as described in **CHAPTER II**. Values are the mean ± SEM n=12 hearts in each group. ^{t}significantly different from comparable hearts at low work ($p \leq 0.05$).

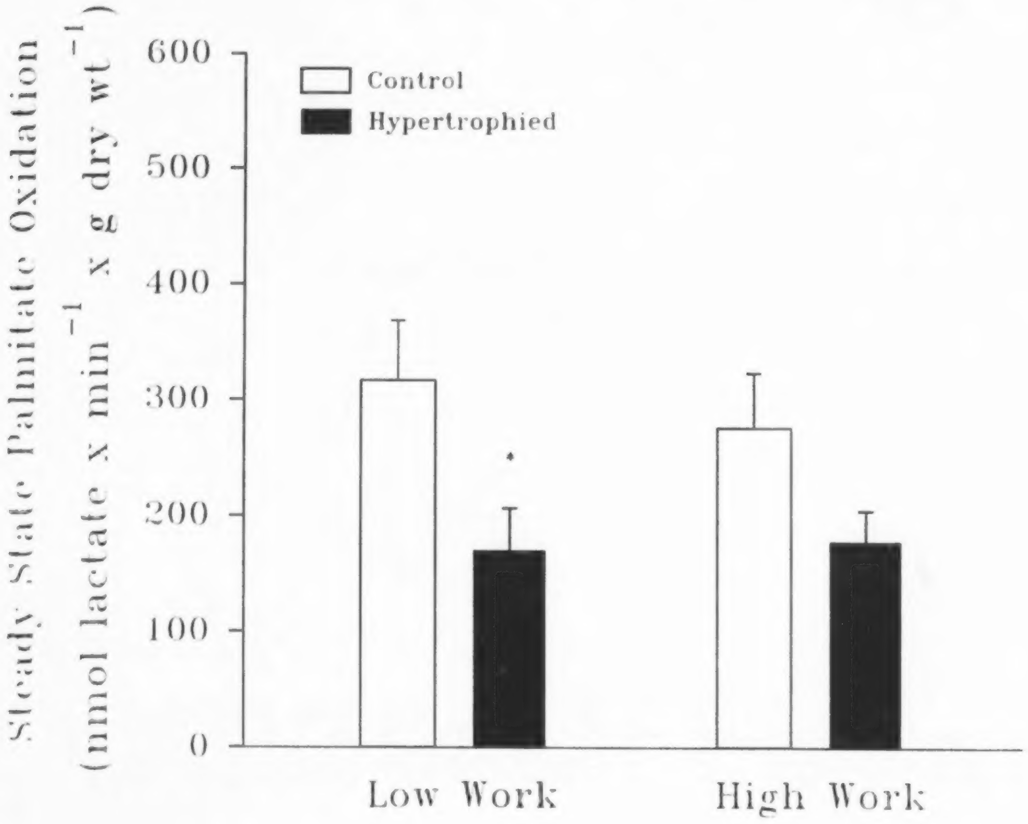

Figure 3-3: Steady state palmitate oxidation in control and hypertrophied rat hearts subjected to low and high work state. Steady state rates of palmitate oxidation were determined in hearts perfused with 11 mM glucose, 0.5 mM lactate, 0.4 mM [9,10-^{3}H]palmitate, and 100 μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of either 60 mm Hg (low work) or 120 mm Hg (high work). ^3H_2O production from [9,10-^{3}H]palmitate was measured as described in **CHAPTER II**. Values are the mean ± SEM n=9 to 11 hearts in each group. *significantly different from control hearts ($p \leq 0.05$).

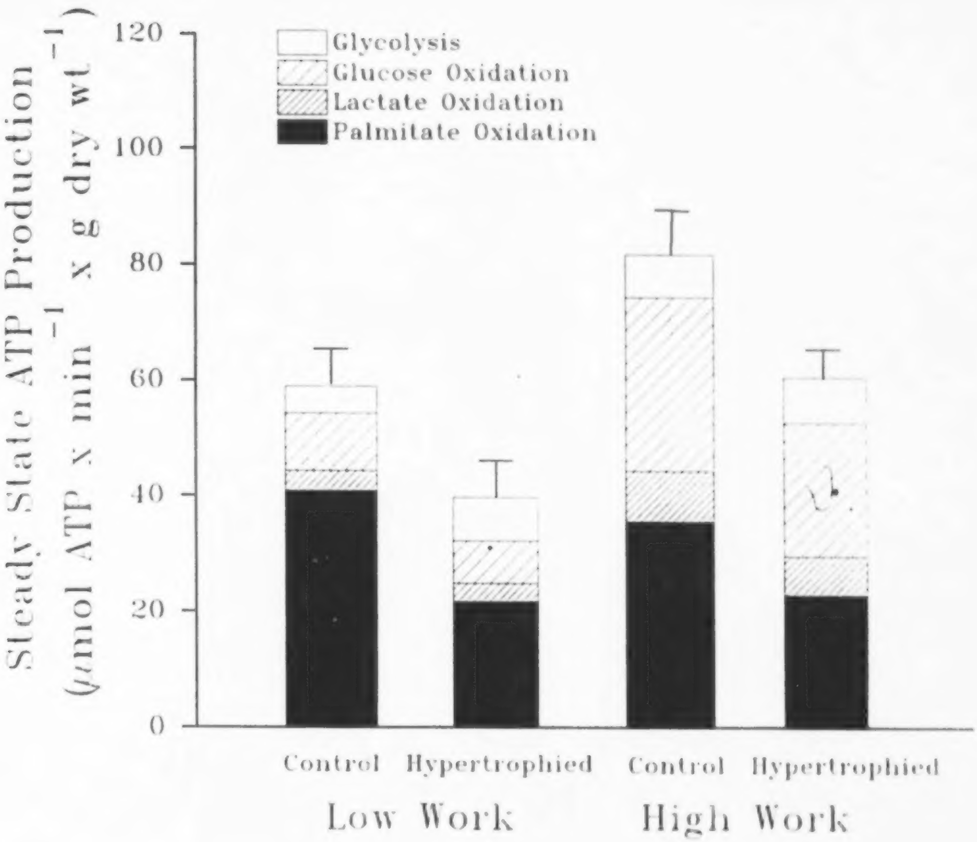

Figure 3-4: Steady state ATP production in control and hypertrophied rat hearts subjected to low and high work states. Hearts were perfused with 11 mM glucose, 0.5 mM lactate, 0.4 mM palmitate, and 100 $\mu U \cdot ml^{-1}$ insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of either 60 mm Hg (low work) or 120 mm Hg (high work). ATP production rates were calculated from the data shown in Figures 3-1 to 3-3 (calculations described in **CHAPTER II**).

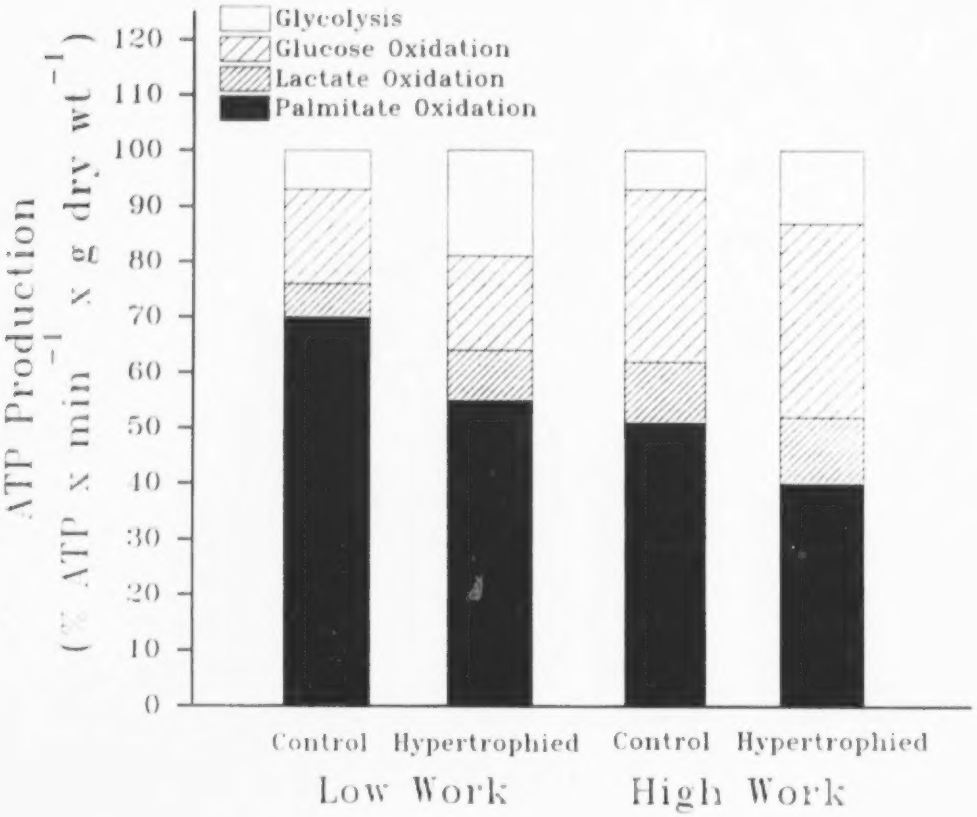

Figure 3-5: Percent ATP production in control and hypertrophied rat hearts subjected to low and high work states . Rates of ATP production from glycolysis, glucose, lactate and palmitate oxidation (Figure 3-4) are expressed as percent contribution of total ATP production.

CHAPTER IV

Competition between exogenous lactate and fatty acids as sources of ATP in the isolated working rat heart

A) Introduction

It is clear from the data presented in **CHAPTER III** that the rate of lactate oxidation is similar between hypertrophied and normal rat hearts. Therefore the following experiments were performed to determine the contribution of lactate and fatty acids to ATP production in the normal isolated working rat heart. A protocol in which the exogenous lactate concentration was increased stepwise over a 100 min period from 0.0 to 8.0 mM lactate was used. The free fatty acid concentration was set at either 0.4 or 1.2 mM palmitate. This allowed for the investigation of a range of conditions which included a physiological state such as 0.5 mM lactate and 0.4 mM palmitate, as well states representing exercise or severe stress (8.0 mM lactate and 1.2 mM palmitate).

B) Methods

i) Animals

The animals used in this study were normal male Sprague-Dawley rats weighing 250-350 g.

ii) Specific perfusion protocol

Following cannulation of the aorta and left atria, the hearts (n=34) were perfused in the working heart mode with a perfusion buffer containing 5.5 mM glucose, 1.25 mM Ca^{2+} and 100 $\mu U \cdot ml^{-1}$ insulin. Alterations in fatty acid content of the perfusion buffer depended on the perfusion series in which either 0.4 or 1.2 mM palmitate was prebound to bovine serum albumin was used. Hearts were perfused for 100 min over which time the lactate concentration was increased every 20 min so that the concentrations of lactate in the perfusion buffer were either 0.0, 0.5, 2.0, 5.0 or 8.0 mM.

One series of perfusions contained either [5-^{3}H/U-^{14}C]glucose to measure glycolysis and glucose oxidation respectively, whereas a parallel series had [U-^{14}C]lactate and [9,10-^{3}H]palmitate in the perfusion buffer to measure lactate and palmitate oxidation respectively (described in **CHAPTER II**). Perfusate buffer samples required for metabolic analysis were taken at 10, 15, 20, 30, 35, 40, 50, 55, 60, 70, 75, 80, 90, 95, 100 min during the perfusion series, with the lactate concentration being increased at 20, 40, 60, and 80 min. Hearts were frozen at the end of the 100 min perfusion period.

Oxygen consumption was measured in a series of hearts (n=24), in which the pulmonary artery was cannulated and the coronary flow directed over a Yellow Springs Instrument Company Incorporated oxygen probe. These hearts were perfused for 100 min with 5.5 mM glucose, 1.25 mM Ca^{2+}, in a Krebs'-Henseleit buffer that contained either 0.4 or 1.2 mM palmitate, and 0.5 or 8.0 mM lactate. Therefore four separate combinations were used for oxygen consumption measurements. These hearts were also frozen at the end of the 100 min perfusion period, and tissue glycogen and triglyceride content was measured.

C) Results

i) Myocardial function of hearts perfused with 0.4 or 1.2 mM palmitate and increasing concentrations of lactate

A summary of the functional data is shown in Table 4-1 (0.4 mM palmitate) and Table 4-2 (1.2 mM palmitate). Mechanical function expressed as HR·PSP is shown in Figure 4-1. As the lactate concentration was increased in the perfusion buffer, what appeared be a dose-dependent depression in mechanical function was observed. After 60 min of perfusion the mechanical function declined and reached statistical significance in both the 0.4 and 1.2 mM palmitate groups at 100 min (8.0 mM lactate). There was no significant difference in the mechanical function of the 0.4 and 1.2 mM palmitate groups at the 100 min time point. Whether or not the lactate was responsible for the depression in mechanical function seen at 100 min compared to 60 min (2.0 mM lactate), and 80 min (5.0 mM lactate) could not be delineated from this series of hearts. Therefore, perfusions were carried out in which the hearts were exposed to either 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate for the entire 100 min period perfusion with all other conditions remaining the same (thus, four additional perfusion series were carried out).

These results, displayed in Figure 4-2, show a similar depression in function over the 100 min period, but now it appears to be a time-dependent phenomenon. All groups, except the 8.0 mM lactate and 1.2 mM palmitate group, showed a significant depression in mechanical function at 100 min compared to 40 min of perfusion. Hearts supplied with 0.4 mM palmitate (and either 0.5 or 8.0 mM lactate) had significantly depressed mechanical function at 100 min compared to hearts supplied with 1.2 mM palmitate (and either 0.5 or 8.0 mM lactate).

ii) Myocardial rates of glycolysis in normal hearts exposed to increasing concentrations of lactate and 0.4 or 1.2 mM palmitate

Glycolytic rates at 0.5 or 8.0 mM lactate, and 0.4 and 1.2 mM palmitate are shown in Figure 4-3. These values for glycolysis (as well as glucose, lactate and palmitate oxidation) are taken from the perfusion series of hearts where the lactate concentration was increased from 0.0 to 8.0 mM (Figure 4-1). In the presence of 0.5 mM lactate there was a slight depression in glycolytic rates in hearts supplied 1.2 mM palmitate compared hearts perfused with 0.4 mM palmitate. At 0.4 mM palmitate, rates of glycolysis were near "maximal" no matter what the lactate concentration was (Table 4-3). As the lactate concentration increased from 0.0 to 8.0 mM in the 1.2 mM palmitate hearts, rates of glycolysis increased stepwise until they were significantly accelerated in the 5.0 and 8.0 mM lactate groups compared to the no lactate group (Table 4-4).

iii) Myocardial rates of glucose oxidation in normal hearts exposed to increasing concentrations of lactate and 0.4 or 1.2 mM palmitate

Glucose oxidation was only mildly affected by increases in both lactate and palmitate in these perfusions (Figure 4-4). At 0.4 mM palmitate glucose oxidation increased slightly, but not significantly as the lactate concentration increased (Table 4-3). At 1.2 mM palmitate, rates of glucose oxidation did not change (Table 4-4). Only at 8.0 mM lactate was a significant depression seen in the rates of glucose oxidation in hearts perfused with 1.2 mM palmitate compared to those perfused with 0.4 mM palmitate.

iv) Myocardial rates of lactate oxidation in normal hearts exposed to increasing concentrations of lactate and 0.4 or 1.2 mM palmitate

Rates of lactate oxidation showed dramatic changes when exogenous lactate supply was increased. Lactate oxidation also changed dramatically by altering exogenous fatty acid supply (Figure 4-5). In the presence of 0.4 mM palmitate the oxidation of lactate increased considerably as the concentration of lactate increased (Table 4-3). An increase in the rates of lactate oxidation also occurred at 1.2 mM palmitate as the lactate concentration increased (Table 4-4).

As described above, increasing the exogenous supply of lactate to 8.0 mM, resulted in a greater than four fold increase in rates of lactate oxidation in the presence of 0.4 mM palmitate (from 1239 ± 236 to 5247 ± 940 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{g dry wt}^{-1}$). In the presence of 1.2 mM palmitate, increases in exogenous lactate also resulted in a dramatic increase in rates of lactate oxidation (from 341 ± 49 to 2535 ± 527 nmol·min^{-1}·g dry wt^{-1}). The presence of 1.2 mM palmitate significantly suppressed lactate oxidation at both 0.5 and 8.0 mM exogenous lactate compared to hearts perfused with 0.4 mM palmitate (Figure 4-5).

v) Myocardial rates of palmitate oxidation in normal hearts exposed to increasing concentrations of lactate and 0.4 or 1.2 mM palmitate

In the presence of 0.4 palmitate rates of palmitate oxidation increased slightly as the lactate concentration increased (Table 4-3). Rates of palmitate oxidation also increased at 1.2 mM palmitate as the concentration of lactate increased (Table 4-4). Figure 4-6 shows rates of palmitate oxidation for the perfusions at 0.5 or 8.0 mM lactate and 0.4 and 1.2 mM palmitate. As shown in Tables 4-3 and 4-4 as well as in Figure 4-6, at a concentration of 0.5 mM lactate, the rate of palmitate oxidation doubled when exogenous palmitate increased from 0.4 to 1.2 mM. A similar phenomenon is seen in the 8.0 mM lactate group; namely, palmitate oxidation increased almost 2-fold from the 0.4 mM palmitate group to the 1.2 mM palmitate group. When the concentration of lactate was increased to 8.0 mM, a slight increase in the rate of palmitate oxidation occurred in the 0.4 mM palmitate group (from 703 ± 79 to 1025 ± 267 nmol·min^{-1}·g dry wt^{-1}). Only in the 1.2 mM palmitate group was this lactate induced increase in exogenous palmitate oxidation statistically significant (from 1462 ± 121 to 2194 ± 196 nmol·min^{-1}·g dry wt^{-1}).

vi) Myocardial rates of ATP production in normal hearts exposed to increasing concentrations of lactate and 0.4 or 1.2 mM palmitate

Total rate of ATP production in hearts perfused with 0.5 or 8.0 mM lactate and 0.4 and 1.2 mM palmitate is shown in Figure 4-7 and percentage of ATP production is shown in Figure 4-8. When hearts were perfused with only 0.4 mM palmitate and 5.5 mM glucose, the majority of ATP comes from exogenous fatty acids (81%). However there is a significant contribution of endogenous triacylglycerols to ATP production under this condition (Saddik and Lopaschuk, 1991). When lactate is added to the perfusion system it supplies about 17% of the ATP from exogenous sources. Furthermore, it can clearly be seen that an increase from 0.5 to 8.0 mM lactate, resulted in a dramatic increase in the contribution of lactate to total rates of ATP synthesis (from 17% to 37%). The contribution of lactate occurred in a stepwise manner as the lactate concentration increased during the perfusion (17%, 30%, 35%, and 37% at 0.5, 2.0, 5.0 and 8.0 mM lactate, respectively). Although lactate does indeed significantly contribute to overall rates of ATP production, it never becomes the primary energy source, even at low (0.4 mM) palmitate.

There was also an increase in the contribution of lactate oxidation to ATP production in the 1.2 mM palmitate group. As seen in Figure 4-7 and Figure 4-8, the contribution of lactate oxidation to overall ATP production increased as the lactate concentration increased from 0.5 to 8.0 mM. A similar stepwise increase in lactate oxidation and its contribution to ATP production occurred at the intermediate concentrations of lactate (3%, 8%, 11%, and 13% at 0.5, 2.0, 5.0, and 8.0 mM lactate, respectively). The end result was similar to what we see at low (0.4 mM) palmitate. The majority of ATP was derived from fatty acids, even at 8.0 mM lactate (90% at 0.5 mM, and 81% at 8.0 mM lactate). Only at 0.4 mM palmitate does lactate make a major contribution towards total ATP production; 17% at 0.5 mM lactate and 37% at 8.0 mM lactate. This compares to 3% at 0.5 mM lactate and 1.2 mM palmitate, and 13% at 8.0 mM lactate and 1.2 mM palmitate.

vii) Myocardial rates of oxygen consumption in normal hearts exposed to increasing concentrations of lactate and 0.4 or 1.2 mM palmitate

Total rates of ATP production increased as the exogenous supply of lactate and palmitate increased (Figure 4-7). Looking at the 0.4 mM palmitate group, there was a 58% increase in total rates of ATP production, as the lactate concentration increased form 0.5 to 8.0 mM. To see if this also resulted in an increased rate of O_2 consumption, O_2 consumption was measured throughout 100 min of perfusion with 0.5 or 8.0 mM lactate and 0.4 or 1.2 mM palmitate. O_2 consumption remained steady over the 100 min perfusion in hearts perfused with 0.5 or 8.0 mM lactate and 1.2 mM palmitate (Figure 4-9). Hearts perfused with 0.5 mM lactate and 0.4 mM palmitate showed a decrease in O_2 consumption past 60 min of perfusion, however this was not statistically significant.

A steady decline in the O_2 consumption was seen in the 8.0 mM lactate and 0.4 mM palmitate group, which was statistically significant when comparing the 10 and 20 min time points to the 80 and 100 min time points. This decline in O_2 consumption in the 0.4 mM palmitate group may be due to the decrease in function seen over the 100 min perfusion. Therefore, to see if this were true O_2 consumption was normalized for mechanical function (Figure 4-10). The result was that over the 100 min perfusion, changes in O_2 consumption could be explained by the changes occurring in mechanical function, because the normalized value did not alter.

In the 0.4 mM palmitate group O_2 consumption in the presence of 0.5 and 8.0 mM lactate was 39.1 ± 4.9 *vs* 44.6 ± 4.0 $\mu mol \cdot min^{-1} \cdot g$ dry wt^{-1}, respectively (40 min time point). Calculating rates of O_2 consumption from steady state rates of glucose, lactate, and palmitate oxidation, the 0.4 mM palmitate group has an O_2 consumption of 22.14 ± 2.03 and 42.39 ± 6.77 $\mu mol \cdot min^{-1} \cdot g$ dry wt^{-1} at 0.5 and 8.0 mM lactate respectively. In the 1.2 mM palmitate group O_2 consumption in the presence of 0.5 and 8.0 mM lactate was 43.2 ± 5.7 *vs* 50.4 ± 13.5 $\mu mol \cdot min^{-1} \cdot g$ dry wt^{-1}, respectively (40 min time point). Again, calculating the rates of O_2 consumption reveals that the rates should be 36.5 ± 2.83 and 59.91 ± 4.8 $\mu mol \cdot min^{-1} \cdot g$ dry wt^{-1} at 0.5 and 8.0 mM lactate respectively. Differences in the calculated rates of O_2 consumption and the actual measured values in the 0.5 mM lactate and 0.4 mM palmitate group may come from the contribution of endogenous carbon sources (glycogen and triacylglycerol) to overall O_2 consumption.

viii) Myocardial triacylglycerol and glycogen content

As stated above, a discrepancy existed in the actual measured rates of O_2 consumption and the calculated rates. To see if there was any change in the triacylglycerol pool at the end of the 100 min period of perfusion at either 0.5 or 8.0 mM lactate and 0.4 and 1.2 mM palmitate, the myocardial triacylglycerol pool was measured. As shown in Table 4-5, myocardial triacylglycerol was significantly lower in the 0.5 mM lactate and 0.4 mM palmitate group compared to the other groups. In light of the difference seen between the calculated rates of O_2 consumption (from ATP production) and the measured rates of O_2 consumption in the 0.5 mM lactate and 0.4 mM palmitate group, the extra O_2 consumed may be due to significant use of triacylglycerol as an ATP source. This has previously been shown to occur under similar conditions of low exogenous palmitate (0.4 mM), where triacylglycerol contributes significantly to ATP production (Saddik and Lopaschuk, 1991).

Another possible carbon source for the production of ATP is glycogen. The glycogen pool was measured at the end of the 100 min period of perfusion. Normal unperfused hearts have a basal glycogen level of 116.1 ± 11.9 (n=8) μmol·g dry wt^{-1}. Table 4-5 shows the glycogen content of hearts at the end of 100 min perfusion with either 0.5 or 8.0 mM lactate and 0.4 or 1.2 mM palmitate. The content of myocardial glycogen was not different among the four perfusion groups. However, it is evident that the tissue content of glycogen did increase over the 100 min perfusion period when compared to the tissue content of unperfused rat hearts.

ix) Myocardial efficiency

When the amount of ATP produced per unit mechanical function performed was calculated, the hearts perfused with 0.4 mM palmitate were more efficient than hearts perfused with 1.2 mM palmitate. At 0.5 mM lactate and 0.4 mM palmitate, 1210 ± 122 nmol ATP$\cdot$min^{-1} are used per unit function (measured as HR$\cdot$PSP$\cdot$10^{-3}). This compares to 1964 ± 114 nmol ATP$\cdot$min$^{-1}\cdot$(HR$\cdot$PSP$\cdot$10^{-3})$^{-1}$ at 0.5 mM lactate and 1.2 mM palmitate. This discrepancy may be due to the contribution of endogenous triglycerides to ATP production, which will be greater in the 0.4 mM palmitate group (Saddik and Lopaschuk, 1992).

In hearts perfused for 100 min with either 0.5 or 8.0 mM lactate and 0.4 or 1.2 mM palmitate, there was no change in the amount of oxygen consumed when normalized per unit mechanical function (Figure 4-10). However, there was an increase in the amount of ATP being produced (Figure 4-7). This would suggest that there is an increased efficiency in the amount of ATP being produced per unit oxygen consumed at the end of the perfusion series.

D) Summary

From this data it can be concluded that;

1) lactate contributes significantly towards ATP production only when the exogenous fatty acid concentration is low (0.4 mM palmitate),

2) when the exogenous supply of fatty acids is high (1.2 mM palmitate), lactate does not contribute nearly as much towards total ATP production,

3) under no circumstances in which a physiologically relevant perfusion buffer is used, can lactate be considered the primary source of ATP for the isolated working rat heart.

Table 4-1: Functional parameters of normal hearts exposed to varying concentrations of lactate and 0.4 mM palmitate

Parameter Measured	0.0 mM lactate	0.5 mM lactate	2.0 mM lactate	5.0 mM lactate	8.0 mM lactate
Heart Rate (beats·min^{-1})	259 ± 14	271 ± 11	272 ± 11	276 ± 11	273 ± 11
Peak Systolic Pressure (mm Hg)	110 ± 2	105 ± 2	$98 \pm 3^{*}$	$89 \pm 3^{*\dagger\S}$	$79 \pm 3^{*\dagger\S\P}$
Developed Pressure (mm Hg)	37 ± 3	$29 \pm 2^{*}$	$24 \pm 2^{*}$	$20 \pm 1^{*\dagger}$	$16 \pm 1^{*\dagger\S}$
HR·PSP (beats·mm Hg^{-1}·10^{-3})	28.4 ± 1.4	28.3 ± 1.2	26.8 ± 1.3	24.6 ± 1.3	$21.6 \pm 1.1^{*\dagger\S}$
HR·dP (beats·mm Hg^{-1}·10^{-3})	9.1 ± 0.5	7.9 ± 0.5	$6.7 \pm 0.5^{*}$	$5.3 \pm 0.4^{*\dagger\S}$	$4.3 \pm 0.4^{*\dagger\S}$

Values are the mean ± SEM, n=15. * significantly different from 0.0 mM lactate ($p \leq 0.05$). † significantly different from 0.5 mM lactate ($p \leq 0.05$). § significantly different from 2.0 mM lactate ($p \leq 0.05$). ¶ significantly different from 5.0 mM lactate ($p \leq 0.05$).

Table 4-2: Functional parameters of normal hearts exposed to varying concentrations of lactate and 1.2 mM palmitate

Parameter Measured	0.0 mM lactate	0.5 mM lactate	2.0 mM lactate	5.0 mM lactate	8.0 mM lactate
Heart Rate (beats·min^{-1})	259 ± 12	273 ± 9	277 ± 10	258 ± 18	232 ± 19
Peak Systolic Pressure (mm Hg)	105 ± 2	104 ± 2	95 ± 5	$84 \pm 7^{*\dagger}$	$73 \pm 7^{*\dagger\S}$
Developed Pressure (mm Hg)	34 ± 2	31 ± 2	$26 \pm 2^{*}$	$20 \pm 2^{*\dagger\S}$	$17 \pm 2^{*\dagger\S}$
HR·PSP (beats·mm Hg^{-1}·10^{-3})	27.0 ± 1.1	28.3 ± 1.0	26.6 ± 1.6	23.5 ± 2.2	$18.5 \pm 2.2^{*\dagger\S\P}$
HR·dP (beats·mm Hg^{-1}·10^{-3})	9.0 ± 0.4	8.3 ± 0.5	$7.3 \pm 0.5^{*}$	$5.4 \pm 0.5^{*\dagger\S}$	$4.2 \pm 0.5^{*\dagger\S}$

Values are the mean ± SEM, n=19. * significantly different from 0.0 mM lactate ($p \leq 0.05$). † significantly different from 0.5 mM lactate ($p \leq 0.05$). § significantly different from 2.0 mM lactate ($p \leq 0.05$). ¶ significantly different from 5.0 mM lactate ($p \leq 0.05$).

Table 4-3: Rates of substrate use in normal hearts exposed to varying concentrations of lactate and 0.4 mM palmitate

Parameter Measured	0.0 mM lactate	0.5 mM lactate	2.0 mM lactate	5.0 mM lactate	8.0 mM lactate
$nmol \cdot min^{-1} \cdot g\ dry\ wt^{-1}$					
Glycolysis	3275 ± 461 (n=7)	3165 ± 460 (n=7)	3408 ± 642 (n=7)	4154 ± 982 (n=6)	3652 ± 807 (n=7)
Glucose Oxidation	387 ± 59 (n=6)	375 ± 96 (n=6)	321 ± 26 (n=6)	550 ± 140 (n=5)	514 ± 39 (n=6)
Lactate Oxidation	NA	1239 ± 236 (n=8)	3376 ± 606† (n=8)	4694 ± 803† (n=8)	5246 ± 940† (n=7)
Palmitate Oxidation	687 ± 50 (n=8)	703 ± 79 (n=6)	952 ± 148 (n=7)	1007 ± 144 (n=6)	1025 ± 267 (n=7)

Values are the mean ± SEM. NA=not applicable. † significantly different from 0.5 mM lactate ($p \leq 0.05$).

Table 4-4: Rates of substrate use in normal hearts exposed to varying concentrations of lactate and 1.2 mM palmitate

Parameter Measured	0.0 mM lactate	0.5 mM lactate	2.0 mM lactate	5.0 mM lactate	8.0 mM lactate
			$nmol\cdot min^{-1}\cdot g\ dry\ wt^{-1}$		
Glycolysis	1565 ± 232 (n=7)	2105 ± 386 (n=6)	2534 ± 672 (n=5)	3249 ± 468 (n=6)	4031 ± 678*† (n=5)
Glucose Oxidation	364 ± 21 (n=5)	312 ± 80 (n=6)	352 ± 72 (n=7)	346 ± 115 (n=3)	307 ± 66 (n=6)
Lactate Oxidation	NA	341 ± 496 (n=12)	1027 ± 124 (n=12)	2103 ± 410†§ (n=12)	2535 ± 527†§ (n=11)
Palmitate Oxidation	1128 ± 108 (n=12)	1462 ± 121 (n=12)	1573 ± 128 (n=12)	2177 ± 115*†§ (n=12)	2194 ± 196*†§ (n=12)

Values are the mean ± SEM, n=15. NA=not applicable. * significantly different from 0.0 mM lactate ($p \leq 0.05$). † significantly different from 0.5 mM lactate ($p \leq 0.05$). § significantly different from 2.0 mM lactate ($p \leq 0.05$).

Table 4-5: Myocardial glycogen and triacylglycerol content of hearts perfused with 0.5 or 8.0 mM lactate and 0.4 or 1.2 mM palmitate

	0.5 mM lactate and 0.4 mM palmitate	0.5 mM lactate and 1.2 mM palmitate	8.0 mM lactate and 0.4 mM palmitate	8.0 mM lactate and 1.2 mM palmitate
μmol·g dry wt^{-1}				
Glycogen Content	155.7 ± 13.4 (n=7)	158.5 ± 16.7 (n=6)	149.9 ± 5.4 (n=6)	165.9 ± 20.8 (n=5)
mg·g dry wt^{-1}				
Triacylglycerol Content	95.6 ± 6.4 (n=6)	153.6 ± 14.1* (n=6)	190.3 ± 16.4* (n=6)	158.0 ± 21.6* (n=5)

* significantly different from 0.4 mM palmitate, 0.5 mM lactate ($p \leq 0.05$).

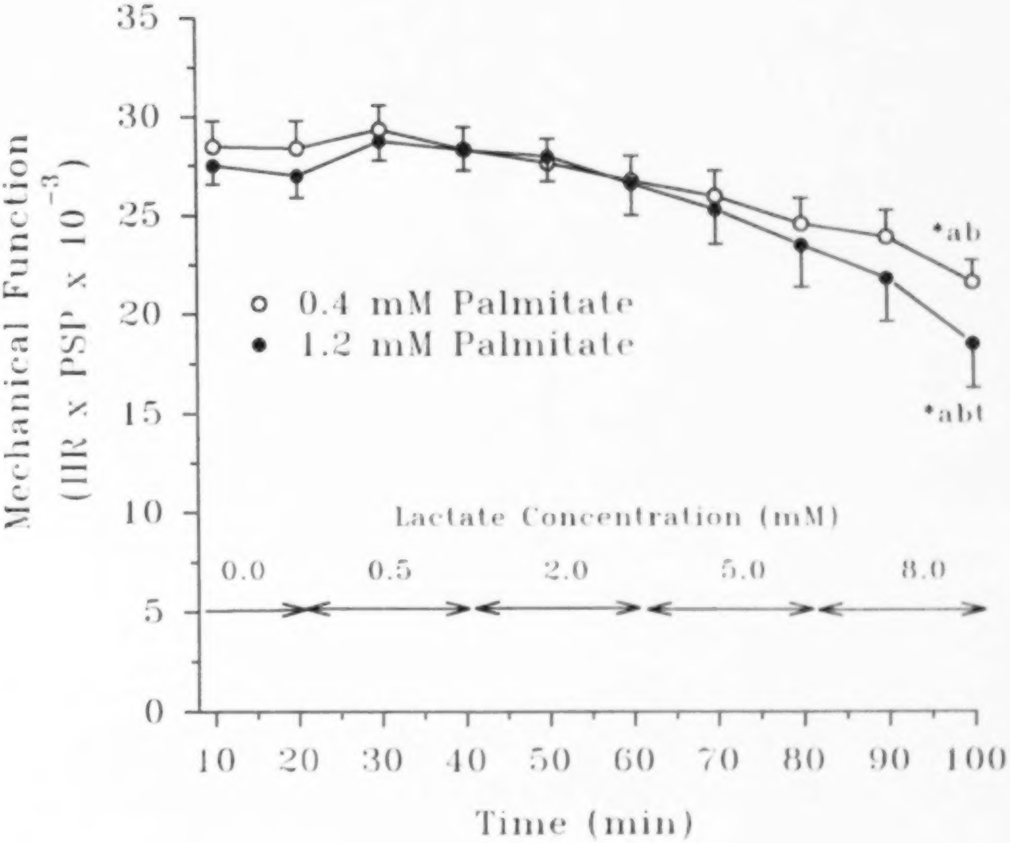

Figure 4-1: Mechanical function of normal rat hearts perfused with increasing concentrations of lactate and 0.4 or 1.2 mM palmitate. Mechanical function is expressed as HR·PSP·10^{-3}. Hearts were perfused with 5.5 mM glucose, 0.0, 0.5, 2.0, 5.0, and 8.0 mM lactate, and 0.4 or 1.2 mM palmitate at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure 80 mm Hg. Values are the mean ± SEM, n=15 (0.4 palmitate group), n=19 (1.2 mM palmitate group). *significantly different from 0.0 mM lactate ($p \leq 0.05$). ^{a}significantly different from 0.5 mM lactate ($p \leq 0.05$). ^{b}significantly different from 2.0 mM lactate ($p \leq 0.05$). ^{t}significantly different from 5.0 mM lactate ($p \leq 0.05$).

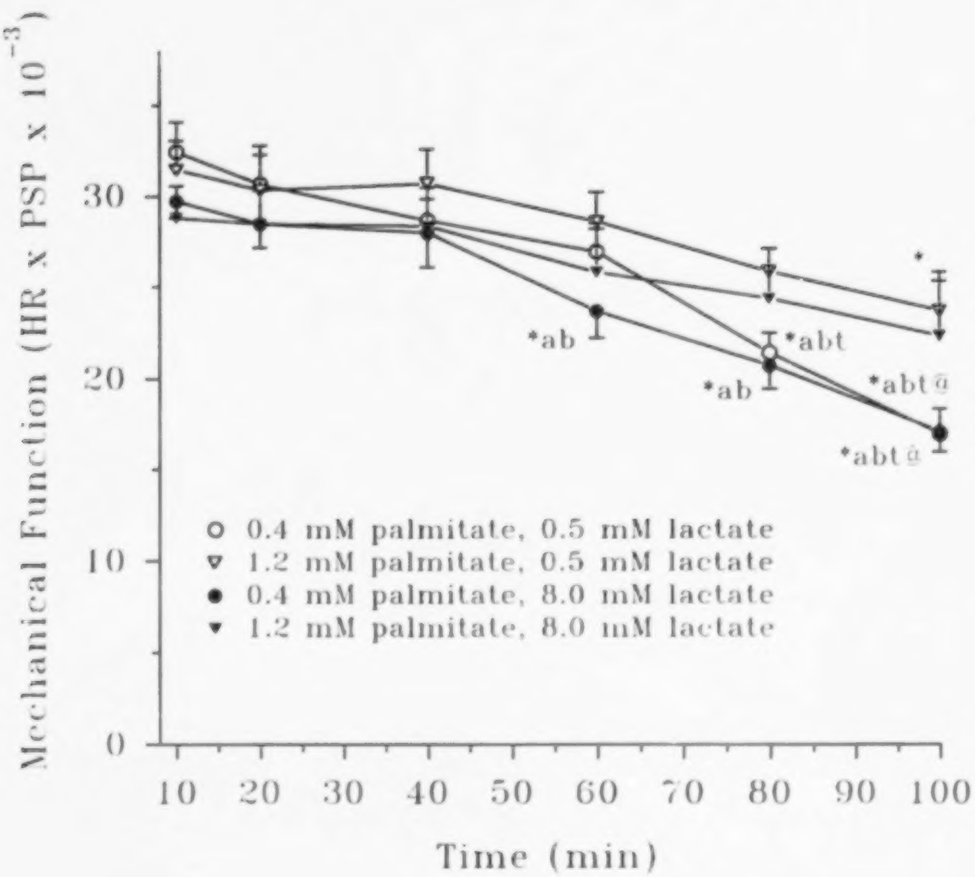

Figure 4-2: Mechanical function of normal rat hearts perfused over 100 min with 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate. Mechanical function is expressed as HR·PSP·10^{-3}. Hearts were perfused with 5.5 mM glucose, 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure 80 mm Hg. Values represent mean ± SEM, n=7 (0.4 mM palmitate and 0.5 mM lactate), n=6 (1.2 mM palmitate and 0.5 mM lactate), n=6 (0.4 mM palmitate and 8.0 mM lactate), and n=5 (1.2 mM palmitate and 8.0 mM lactate). [*]significantly different from 10 min time point ($p \leq 0.05$). [a]significantly different from 20 min time point ($p \leq 0.05$). [b]significantly different from 40 min time point ($p \leq 0.05$). [t]significantly different from 60 min time point ($p \leq 0.05$). [@]significantly different from 1.2 mM palmitate group ($p \leq 0.05$).

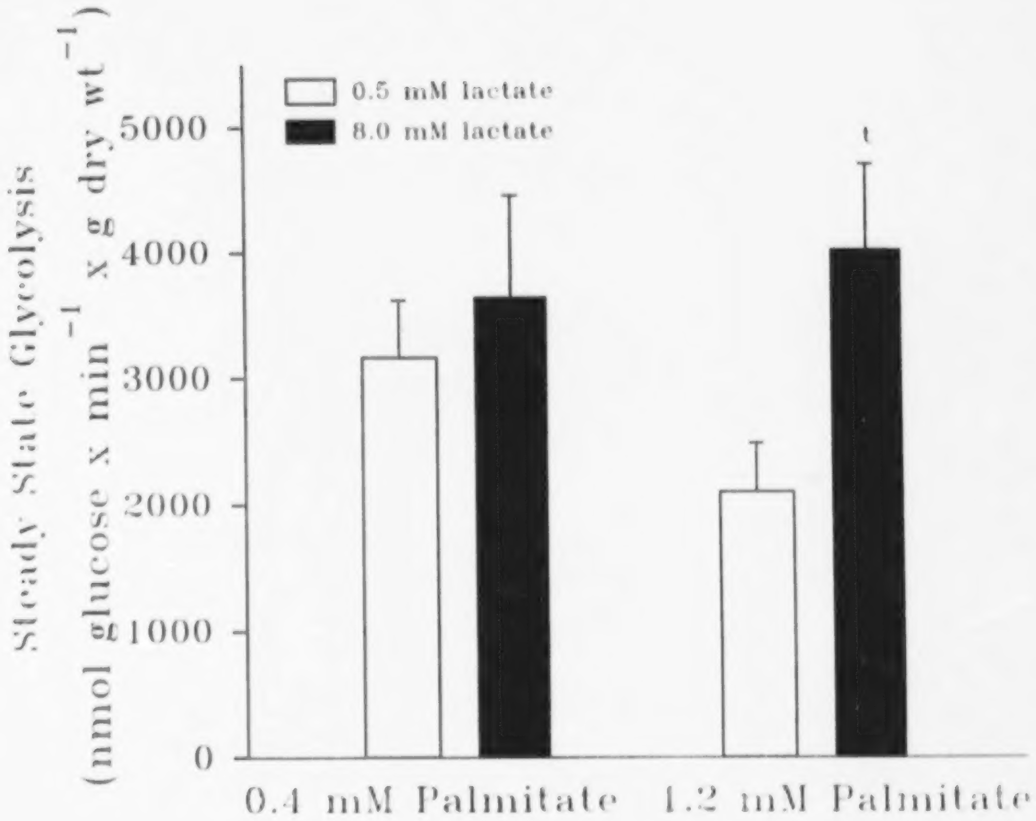

Figure 4-3: Steady state rates of glycolysis in normal rat hearts perfused with 0.5 or 8.0 mM lactate and 0.4 or 1.2 mM palmitate. Steady state rates of glycolysis were determined in hearts perfused with buffer containing 5.5 mM [5-^{3}H]glucose, either 0.5 or 8.0 mM lactate, and either 0.4 or 1.2 mM palmitate at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ^3H_2O production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=7 (0.5 and 8.0 mM lactate and 0.4 mM palmitate) and n=5-6 (0.5 and 8.0 mM lactate and 1.2 mM palmitate). †significantly different from 0.5 mM lactate group ($p \leq 0.05$).

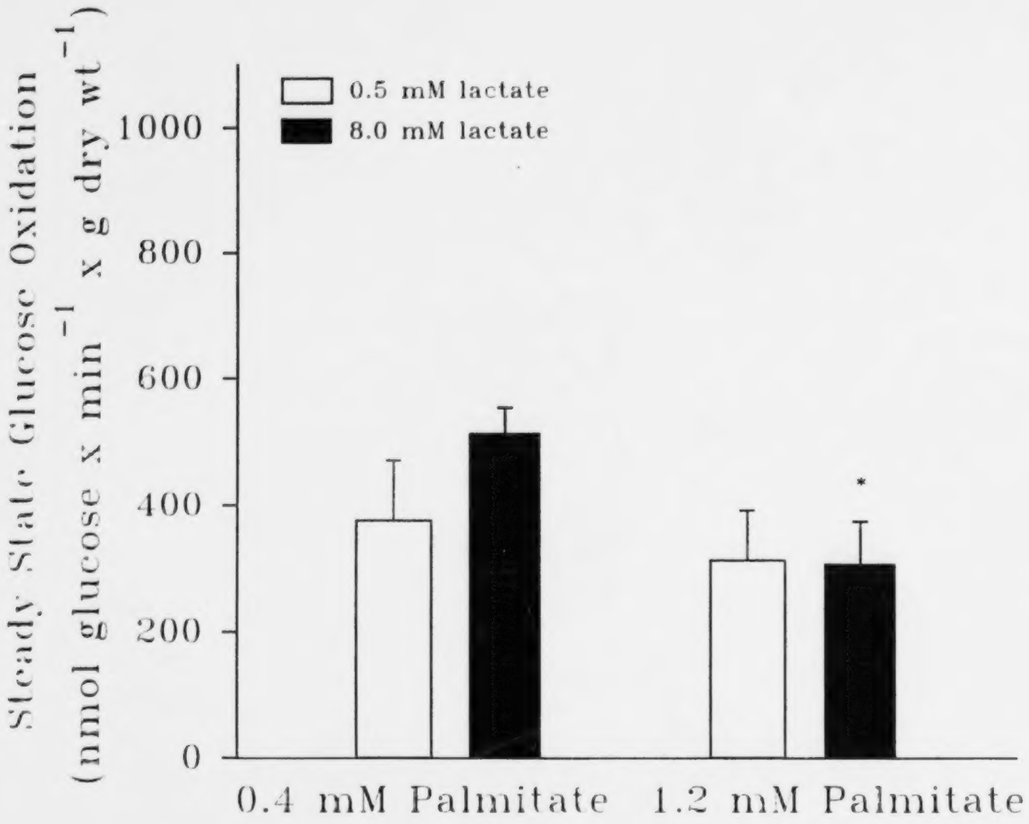

Figure 4-4: Steady state rates of glucose oxidation in normal rat hearts perfused with 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate. Steady state rates of glucose oxidation were determined in hearts perfused with buffer containing 5.5 mM [U-^{14}C]glucose, either 0.5 or 8.0 mM lactate, and either 0.4 or 1.2 mM palmitate at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. $^{14}CO_2$ production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=6 for all groups. *significantly different from 0.4 mM palmitate group ($p \leq 0.05$).

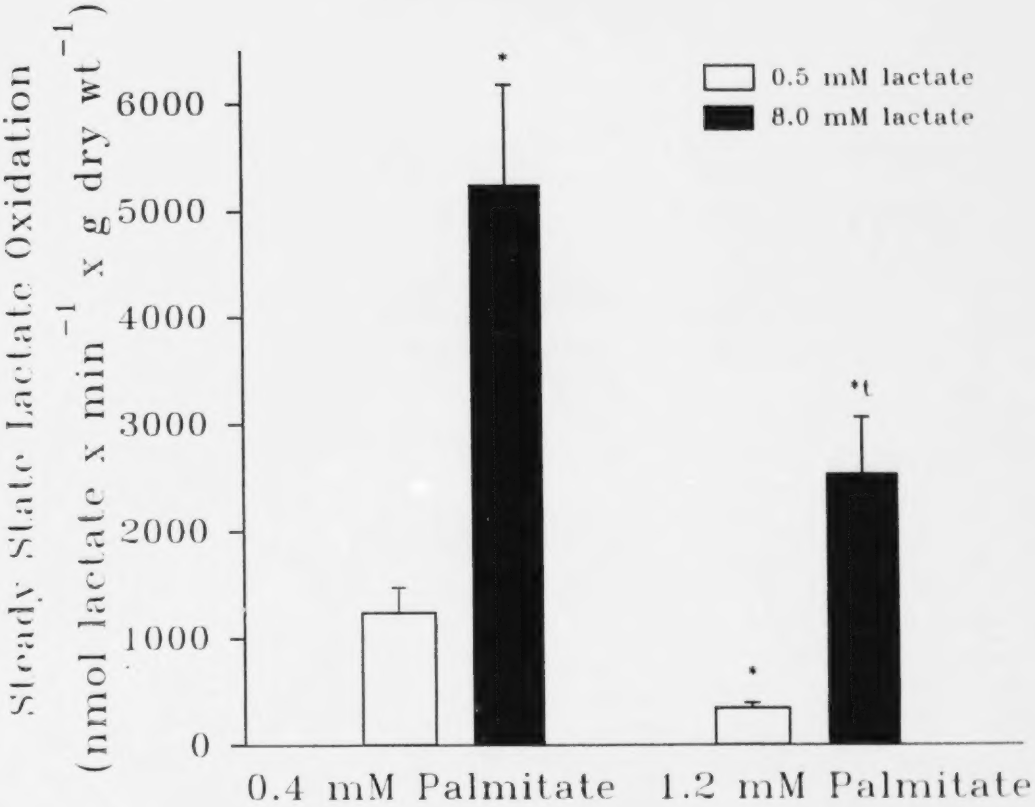

Figure 4-5: Steady state rates of lactate oxidation in normal rat hearts perfused with 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate. Steady state rates of lactate oxidation were determined in hearts perfused with buffer containing 5.5 mM glucose, either 0.5 or 8.0 mM [U-^{14}C]lactate, and either 0.4 or 1.2 mM palmitate at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. $^{14}CO_2$ production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=7-8 (0.5 and 8.0 mM lactate and 0.4 mM palmitate), n=11-12 (0.5 and 8.0 mM lactate and 1.2 mM palmitate). *significantly different from 0.4 mM palmitate group ($p \leq 0.05$). ^{t}significantly different from 0.5 mM lactate group ($p \leq 0.05$).

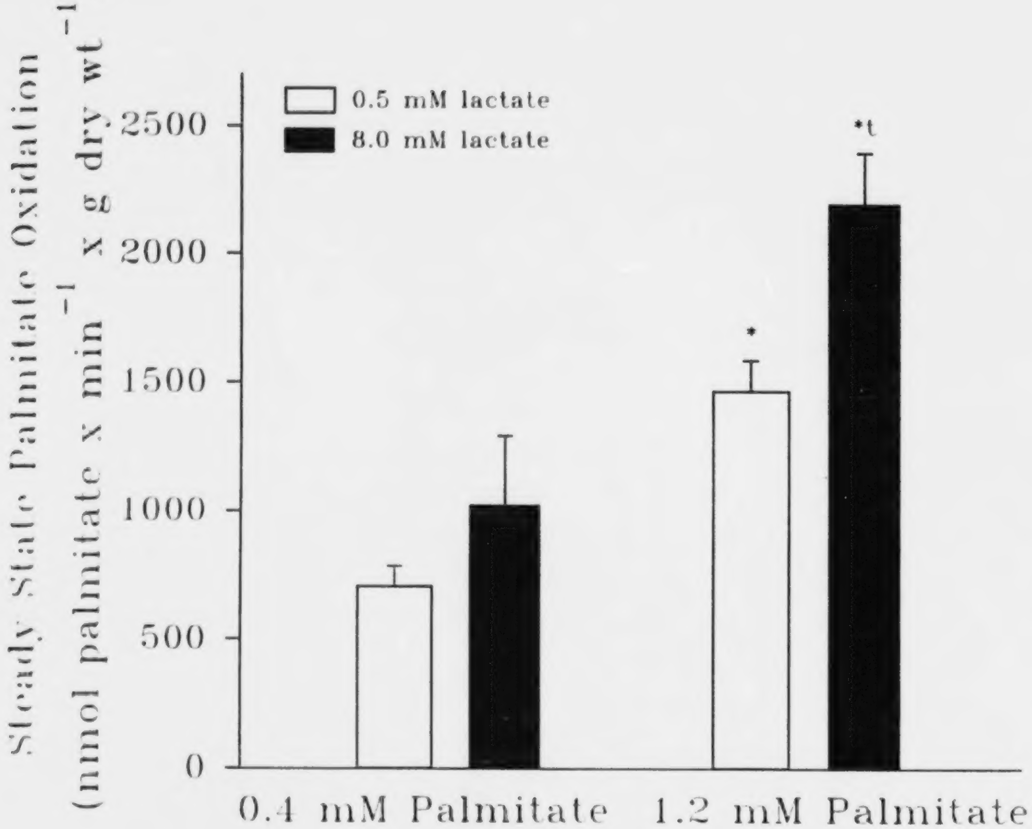

Figure 4-6: Steady state rates of palmitate oxidation in normal rat hearts perfused with 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate. Steady state rates of palmitate oxidation were determined in hearts perfused with buffer containing 5.5 mM glucose, either 0.5 or 8.0 mM lactate, and either 0.4 or 1.2 mM [9,10-^{3}H]palmitate at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ^3H_2O production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=6-7 (0.5 and 8.0 mM lactate and 0.4 mM palmitate), n=12 (0.5 and 8.0 mM lactate and 1.2 mM palmitate). *significantly different from 0.4 mM palmitate group ($p \leq 0.05$). ^{t}significantly different from 0.5 mM lactate group ($p \leq 0.05$).

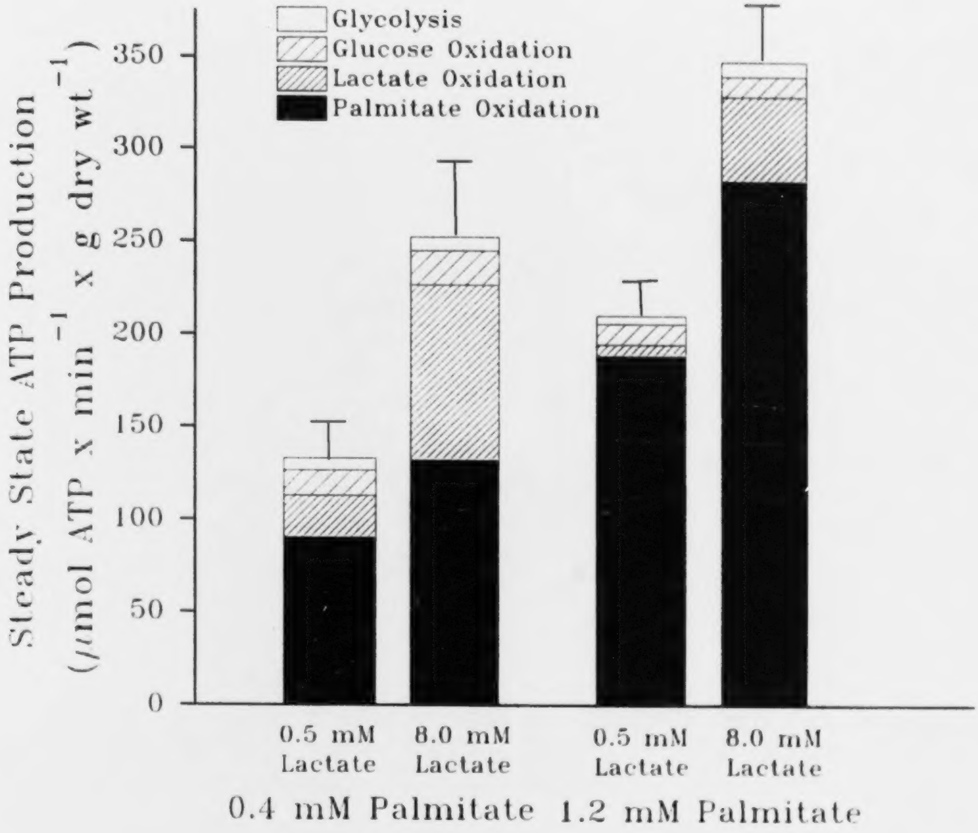

Figure 4-7: Steady state ATP production in normal rat hearts perfused with 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate. ATP production rates were calculated from the data shown in Figures 4-3 to 4-6 (calculations described in **CHAPTER II**).

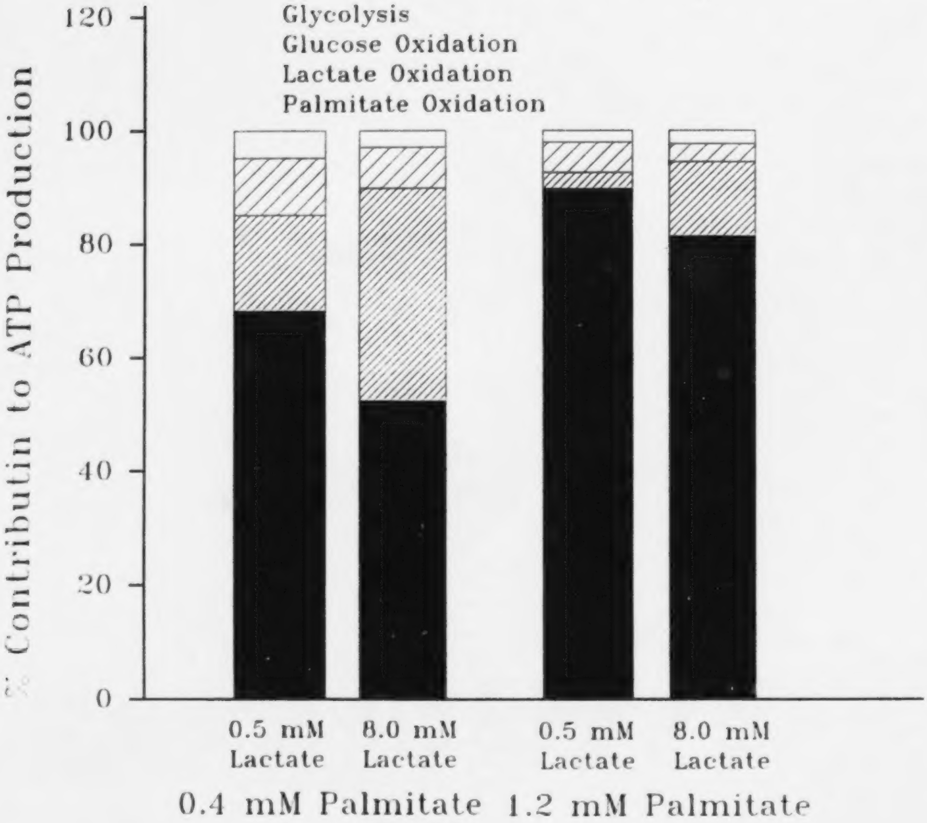

Figure 4-8: Percent ATP production in normal rat hearts perfused with 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate. Rates of ATP production from glycolysis, glucose, lactate and palmitate oxidation (Figure 4-7) are expressed as percent contribution of total ATP production.

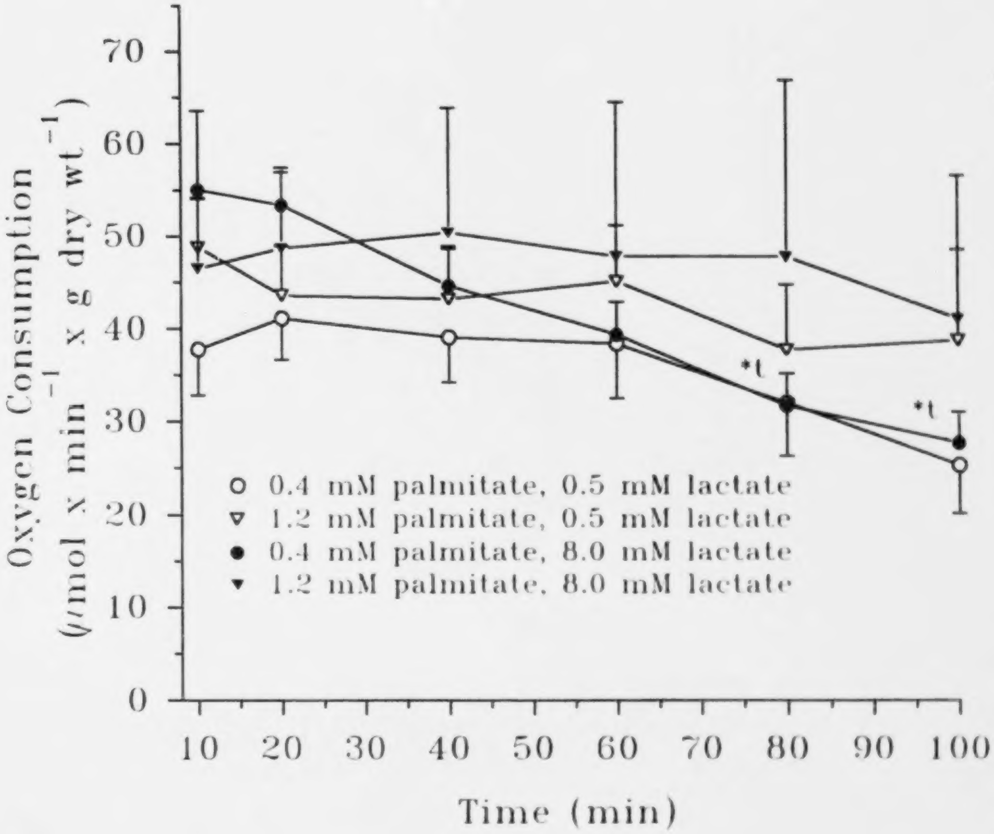

Figure 4-9: Oxygen consumption of normal rat hearts perfused over 100 min with 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate. Hearts were perfused with 5.5 mM glucose, 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure 80 mm Hg. Oxygen consumption was measured as described in **CHAPTER II**. Values represent mean ± SEM, n=7 (0.4 mM palmitate and 0.5 mM lactate), n=6 (1.2 mM palmitate and 0.5 mM lactate), n=6 (0.4 mM palmitate and 8.0 mM lactate), and n=5 (1.2 mM palmitate and 8.0 mM lactate). *significantly different from 10 min time point ($p \leq 0.05$). ᵗsignificantly different from 20 min time point ($p \leq 0.05$).

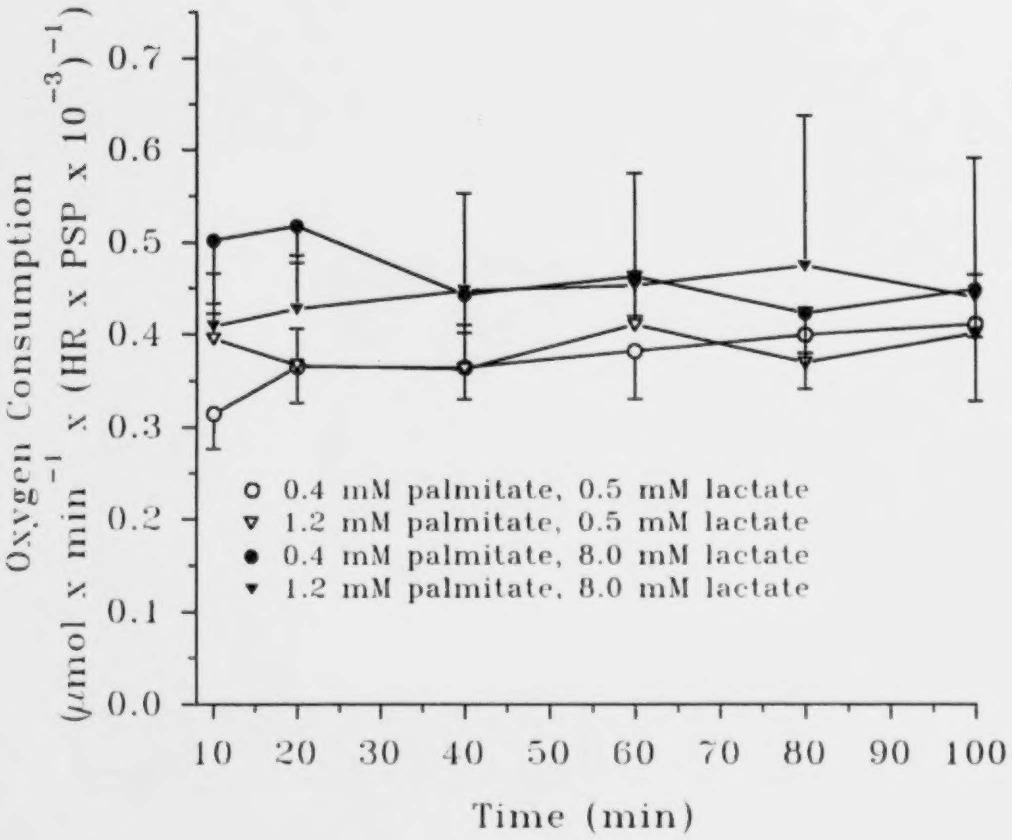

Figure 4-10: Oxygen consumption normalized for mechanical function of normal rat hearts perfused over 100 min with 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate. Hearts were perfused with 5.5 mM glucose, 0.5 or 8.0 mM lactate, and 0.4 or 1.2 mM palmitate at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure 80 mm Hg. Values represent mean ± SEM, n=7 (0.4 mM palmitate and 0.5 mM lactate), n=6 (1.2 mM palmitate and 0.5 mM lactate), n=6 (0.4 mM palmitate and 8.0 mM lactate), and n=5 (1.2 mM palmitate and 8.0 mM lactate).

CHAPTER V

Contribution of glycolysis and oxidative metabolism in hypertrophied rat hearts during post-ischemic reperfusion[8]

A) Introduction

A pressure-overload myocardial hypertrophy increases the severity of mechanical functional abnormalities seen during post-ischemic and post-hypoxic reperfusion (Menasche et al., 1985; Anderson et al., 1987, 1990; Cunningham et al., 1990a; Gaasch et al., 1990). An altered energy substrate metabolism has been implicated as a contributing factor (Menasche et al., 1985; Anderson et al., 1987, 1990a; Cunningham et al., 1990), with a decreased capacity for glycolysis in the hypertrophied heart being suggested to contribute to contractile dysfunction during reperfusion of hearts following hypoxia (Cunningham et al., 1990). However, controversy exists as to whether glycolytic capacity is decreased in the hypertrophied heart (Bishop and Aultschuld 1971; Anderson et al., 1990a; Cunningham et al., 1990). While Cunningham *et al* (1990) have suggested that glycolytic capacity is impaired in hypertrophied hearts, data presented in **CHAPTER III** shows that rates of glycolysis under aerobic conditions are actually significantly increased in hypertrophied hearts compared to normal hearts (Figure 3-1).

It is of paramount importance that oxidative metabolism recovers during post-ischemic reperfusion, so that ATP can be produced for mechanical function and the maintenance of normal ion membrane gradients. In non-hypertrophied hearts, fatty acid oxidation predominates as a source of energy in the post-ischemic period, with overall oxidative metabolism quickly recovering following short periods of ischemia (Lopaschuk et al., 1990, Saddik and Lopaschuk, 1992). In hypertrophied hearts fatty acid oxidation can be depressed even in the absence of ischemia, possibly due to a decrease in myocardial L-carnitine content (El Alaoui-Talibi et al., 1992; Figure 3-3). Cheikh *et al* (1994) have suggested that the hypertrophied heart is in a state of energy starvation, as evidenced by a decreased intramitochondrial NADH/NAD$^+$ ratio. Whether the recovery of carbohydrate or fatty acid oxidation in the hypertrophied heart is compromised following ischemia has not been determined.

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[8] A version of this chapter has been accepted for publication. Schönekess BO, Allard MF, and Lopaschuk GD. 1995. Am J Physiol.

The purpose of this study was to determine if hypertrophied hearts have an altered ability to recover glycolysis and oxidative metabolism during aerobic post-ischemic reperfusion. This was determined in isolated working hearts, both normal and hypertrophied, where hearts are aerobically reperfused following a 30 min period of global no-flow ischemia. Rates of glycolysis, glucose oxidation, lactate oxidation and fatty acid oxidation are directly measured in both the pre-and post-ischemic periods.

B) Methods

i) Hypertrophy model

Myocardial hypertrophy was produced in male Wistar-Kyoto rats as described in **CHAPTER II**.

ii) Specific perfusion protocol

Following cannulation of the aorta and left atria, the hearts were perfused in the working heart mode with a perfusion buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate bound to 3% bovine serum albumin, 2.50 mM Ca^{2+} and 100 $\mu U \cdot ml^{-1}$ insulin. The time course for perfusion was 60 min aerobic working heart, followed by a Langendorff perfusion for 5 min to change the perfusion buffer to a buffer that contained appropriate radiolabelled substrates for the measurement of glycolysis, glucose, lactate, and palmitate oxidation (described in **CHAPTER II**). Glycolysis, glucose oxidation, lactate oxidation and fatty acid oxidation was measured in working hearts during a subsequent 30 min aerobic perfusion. Following this 30 min aerobic period, was a 30 min period of global no-flow ischemia. This was followed by 40 min of aerobic reperfusion. Total perfusion time including ischemia was 2 hr 45 min. Perfusate buffer samples required for metabolic analysis were taken at 10 min intervals during both the pre-ischemic and post-ischemic reperfusion period. Hearts were frozen at the end of the 40 min post-ischemic reperfusion period. As well a separate series of hearts was frozen at the end of ischemia for the measurement of metabolites.

C) Results

i) Heart weight & body weight of experimental animals

Table 5-1 shows the body and wet heart weights of control and aortic banded animals eight weeks after the banding procedure was performed. There was a mild increase in absolute heart weight of aortic banded compared to control hearts (15%). This increase in heart weight was not accompanied by an increase in body weight after eight weeks. As a result, the aortic-banded animals had a significantly increased heart weight to body weight ratio compared to sham operated control animals (16%).

ii) Myocardial function of control and hypertrophied rat hearts

Table 5-2 summarizes pre- and post-ischemic functional measurements taken just prior to the onset of ischemia (i.e., at 30 min of perfusion with radiolabelled buffer) and after 40 min of post-ischemic reperfusion. Control and hypertrophied hearts had a similar mechanical function prior to the ischemic period, measured as HR·PSP·10^{-3}. A slight increase in the PSP and dP (measured as the difference between PSP and the diastolic pressure) was seen in hypertrophied hearts compared to control hearts. During reperfusion following ischemia mechanical function of control hearts recovered to a greater extent than hypertrophied hearts during post-ischemic reperfusion (Figure 5-1). A 71% and 64% recovery of HR·PSP and HR·dP, occurred respectively in control; 40% and 30% of HR·PSP and HR·dP, respectively in the hypertrophied hearts.

iii) Rates of glycolysis and glucose oxidation during pre- and post-ischemic reperfusion of control and hypertrophied hearts

The recovery of glycolysis and glucose oxidation during the 40 min period of aerobic post-ischemic reperfusion is shown in Figures 5-2 and 5-3, respectively. In both cases rates of glycolysis and glucose oxidation (measured as the slope) were constant between 10 and 40 min of reperfusion. Neither glycolysis or glucose oxidation was impaired during post-ischemic reperfusion of hypertrophied hearts.

Steady state rates of glycolysis and glucose oxidation are shown in Figure 5-4 for both the pre-ischemic period and during post-ischemic reperfusion. All steady state rates during post-ischemic reperfusion were determined between 10 and 40 min of reperfusion which is a linear period of glucose metabolism (Figures 5-2 and 5-3). In hypertrophied hearts, pre-ischemic rates of glycolysis were almost two-fold greater than rates of glycolysis in control hearts (4325 ± 716 *vs* 2313 ± 272 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{g dry wt}^{-1}$, respectively). In both hypertrophied and control hearts, the rates of glycolysis were also substantially higher than rates of glucose oxidation, which is consistent with previous studies (Saddik and Lopaschuk, 1991). In hypertrophied hearts rates of glucose oxidation were significantly lower in the pre-ischemic period compared to control hearts (137 ± 25 *vs* 217 ± 26 nmol·min^{-1}·g dry wt^{-1}). This, combined with the higher glycolytic rates, produced a greater imbalance between glycolysis and glucose oxidation in hypertrophied hearts compared to controls.

iv) Rates of lactate oxidation during pre- and post-ischemic reperfusion of control and hypertrophied hearts

The recovery of lactate oxidation during the 40 min period of aerobic post-ischemic reperfusion is shown in Figure 5-5. Rates of lactate oxidation were constant between 10 and 40 min of reperfusion and similar to control hearts, suggesting that lactate oxidation was not impaired during post-ischemic reperfusion of hypertrophied hearts.

Figure 5-6 shows the steady state rates of lactate oxidation in both the pre- and post-ischemic periods. There were no differences between control and hypertrophied hearts during either period. An important observation is that lactate was being taken up and oxidized even though there was a net production of lactate by the heart (based on differences between rates of glycolysis and glucose oxidation) in the control and hypertrophied hearts.

v) Rates of palmitate oxidation during pre- and post-ischemic reperfusion of control and hypertrophied hearts

The recovery of palmitate oxidation during the 40 min period of aerobic post-ischemic reperfusion is shown in Figure 5-7. Rates of palmitate oxidation were constant between 10 and 40 min of reperfusion and similar to control hearts, suggesting that palmitate oxidation was not impaired during post-ischemic reperfusion of hypertrophied hearts.

Steady state rates of fatty acid oxidation are shown in Figure 5-8. In the presence of the high concentrations of fatty acids used in this study the hypertrophied heart does not have altered rates of fatty acid oxidation during either the pre- or post-ischemic reperfusion period, when compared to control hearts. It is also clear that the hypertrophied heart fully recovered rates of palmitate oxidation during the post-ischemic reperfusion period, even though the mechanical function of these hearts remained depressed.

vi) H^+ production from glycolysis uncoupled from glucose oxidation in control and hypertrophied hearts during pre- and post-ischemic reperfusion

During the post-ischemic reperfusion period, rates of glycolysis remain elevated in the hypertrophied hearts compared to control hearts (3533 ± 798 *vs* 1756 ± 443 $nmol \cdot min^{-1} \cdot g$ dry wt^{-1}, respectively), with glucose oxidation actually accelerating in the hypertrophied hearts (from 137 ± 25 to 294 ± 54 nmol·min-1·g dry wt^{-1}). However, the greater imbalance between glycolysis and glucose oxidation in hypertrophied hearts compared to control hearts remained during reperfusion.

The imbalance between glucose oxidation and glycolysis leads to the production of H^+ ions from glycolytically derived ATP (Lopaschuk et al., 1993; Opie, 1990). If a molecule of glucose passes through glycolysis to form 2 pyruvate and these in turn are oxidized (i.e., in the TCA cycle), the net result is a H^+ neutral process when the resulting 38 ATP are hydrolysed. However if glucose only passes through glycolysis and the pyruvate is reduced to lactate, the process results in the production of 2 ATP which are hydrolysed to produce 2 H^+ (Opie, 1990). This scheme is shown in Figure 5-9.

When rates of H^+ production were calculated (Figure 5-10), rates in hypertrophied hearts during pre-ischemic aerobic perfusion were significantly higher than controls (8343 ± 1437 *vs* 4254 ± 519 nmol·min^{-1}·g dry wt^{-1}, respectively). This increase in H^+ production was also present during the post-ischemic reperfusion period in the hypertrophied hearts compared to controls (6442 ± 1562 *vs* 3016 ± 863 nmol·min^{-1}·g dry wt^{-1}, respectively).

vii) Steady state rates of ATP production in control and hypertrophied hearts during pre- and post-ischemic reperfusion

Steady state rates of ATP production (Figure 5-11) and percentage contribution to total ATP production (Figure 5-12) are calculated as described in **CHAPTER II**.

Upon post-ischemic reperfusion the rates of ATP production exceed pre-ischemic values, indicating that oxidative metabolism recovered fully (Figure 5-11). The remainder of the ATP produced came from carbohydrate oxidation (11% and 9%, respectively) and glycolysis (5% and 9%, respectively). The ATP profile was similar between hypertrophied hearts and control hearts with the majority of the ATP produced coming from palmitate oxidation (84% in controls and 82% in hypertrophied hearts) (Figure 5-12).

With the recovery of oxidative metabolism occurring to over 100% of pre-ischemic values, it was surprising to see that the hypertrophied heart does not recover mechanical function during post-ischemic reperfusion (Figure 5-1). When cardiac efficiency is calculated (Mechanical function x Steady State ATP Production^{-1}) during the pre-ischemic period, both the control and hypertrophied hearts had similar values (0.29 *vs* 0.27 HR·PSP·(μmol ATP·min^{-1}·g dry wt^{-1})$^{-1}$, respectively). However during post-ischemia reperfusion, cardiac efficiency decreased dramatically in the hypertrophied heart (from 0.27 to 0.08 HR·PSP·10^{-3}·(μmol ATP·min^{-1}·g dry wt^{-1})$^{-1}$) compared to control hearts (from 0.29 to 0.17 HR·PSP·10^{-3}·(μmol ATP·min^{-1}·g dry wt^{-1})$^{-1}$).

viii) Metabolite content of control and hypertrophied hearts after ischemia and at the end of post-ischemic reperfusion

At the end of the ischemic period there was no difference in either creatine phosphate (4.6 ± 1.1 (n=8) *vs* 5.0 ± 0.4 (n=7) μmol·g dry wt^{-1}) or ATP content (2.5

± 0.3 (n=8) *vs* 2.2 ± 0.5 (n=9) μmol·g dry wt^{-1}) in the control and hypertrophied hearts respectively. At the end of reperfusion creatine phosphate content was increased in both control and hypertrophied hearts (27.3 ± 2.0 (n=9) *vs* 31.8 ± 3.0 (n=8) μmol·g dry wt^{-1}, respectively). ATP content actually recovered to a greater extent in hypertrophied hearts compared to control hearts during reperfusion (9.1 ± 0.7 (n=8) *vs* 5.0 ± 0.6 (n=9) μmol·g dry wt^{-1}, respectively).

Analysis of tissue glycogen content at the end of ischemia showed no difference between the control and hypertrophied hearts (71.3 ± 9.9 (n=9) *vs* 74.7 ± 9.2 (n=8) μmol·g dry wt^{-1}, respectively). The glycogen pool decreased in the hypertrophied heart at the end of reperfusion (56.5 ± 9.9 (n=8) μmol·g dry wt^{-1}), with no change occurring in control hearts. Tissue lactate at the end of ischemia was not different between control and hypertrophied hearts (60.0 ± 7.5 (n=7) *vs* 53.4 ± 5.3 (n=7) μmol·g dry wt^{-1}, respectively). Upon reperfusion, tissue lactate decreased due to restoration of coronary flow. Similar to the end of ischemia, there was no difference in the lactate content of control and hypertrophied hearts at the end of the 40 min period of post-ischemic reperfusion (5.6 ± 1.1 (n=8) *vs* 8.0 ± 1.3 (n=6) μmol·g dry wt^{-1}, respectively).

D) Summary

Evidence presented in this chapter shows that;

1) the hypertrophied heart recovers its ability to oxidize glucose, lactate and palmitate during post-ischemic reperfusion recovery similar to what is seen in control

hearts and mechanical function remains depressed despite the recovery of ATP production,

2) the ability to recruit glycolysis as an ATP source is not impaired in the hypertrophied heart, and in fact rates of glycolysis remain accelerated to a similar extent seen during the normal aerobic perfusion of hypertrophied hearts (i.e., pre-ischemia),

3) H^+ production is similar during post-ischemic reperfusion compared to pre-ischemic aerobic perfusion in hypertrophied hearts.

Table 5-1: Heart and body weight of control and aortic banded animals

	Dry Heart Weight (g)	Wet Heart Weight (g)	Body Weight (g)	Heart Weight/Body Weight Ratio (x 10^{-3})
Control (n=24)	0.34 ± 0.02	1.62 ± 0.05	319 ± 3	5.1 ± 0.2
Hypertrophied (n=24)	0.41 ± 0.02*	1.86 ± 0.06*	315 ± 3	5.9 ± 0.2*

* significantly different from control value ($p \leq 0.05$).

Table 5-2: Functional parameters of control and hypertrophied hearts pre- and post-ischemia

Parameter Measured	Pre-Ischemia		Post-Ischemia	
	Control (n=15)	Hypertrophied (n=18)	Control (n=15)	Hypertrophied (n=18)
Heart Rate (beats·min^{-1})	234 ± 12	210 ± 12	$194 \pm 17^{*}$	$114 \pm 20^{*\dagger}$
Peak Systolic Pressure (mm Hg)	114 ± 4	$126 \pm 4^{*}$	$97 \pm 6^{\dagger}$	$73 \pm 12^{\dagger}$
Developed Pressure (mm Hg)	42 ± 4	$60 \pm 2^{*}$	32 ± 4	$27 \pm 6^{\dagger}$
HR·PSP (beats·mm Hg^{-1}·10^{-3})	27 ± 1	26 ± 1	$18 \pm 2^{\dagger}$	$10 \pm 2^{*\dagger}$
HR·dP (beats·mm Hg^{-1}·10^{-3})	9 ± 1	$12 \pm 1^{*}$	$6 \pm 1^{\dagger}$	$4 \pm 1^{\dagger}$

Pre-ischemia values were taken immediatly prior to ischemia (i.e., 1 hr 35 min into perfusion). Post-ischemia values were taken at the end of the 40 min post-ischemic reperfusion period (i.e., 2 hr 45 min into perfusion).

[*] significantly different from control value ($p \leq 0.05$).

[†] significantly different from comparable pre-ischemic value ($p \leq 0.05$).

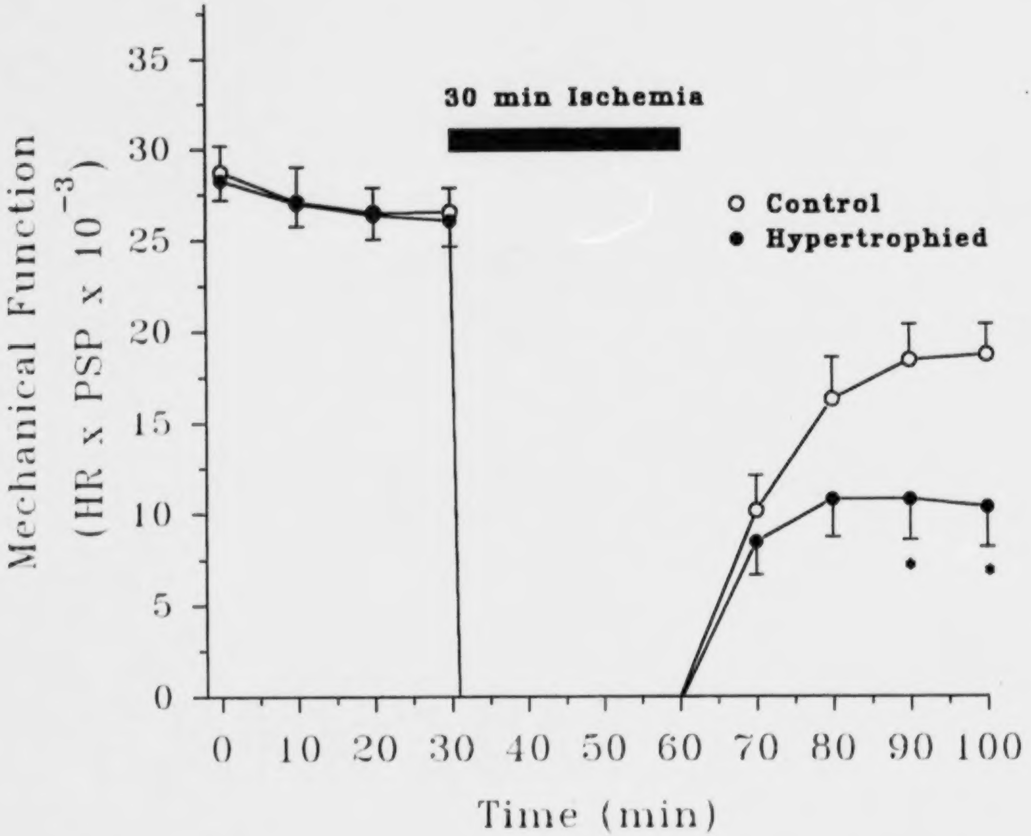

Figure 5-1: Mechanical function of control and hypertrophied rat hearts during pre-ischemic perfusion and post-ischemic reperfusion. Mechanical function is expressed as HR·PSP·10^{-3}. Hearts were perfused with 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100 $\mu U \cdot ml^{-1}$ insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure 80 mm Hg. Values are the mean ± SEM, n=15 (controls), n=18 (hypertrophied). *significantly different from control hearts ($p \leq 0.05$).

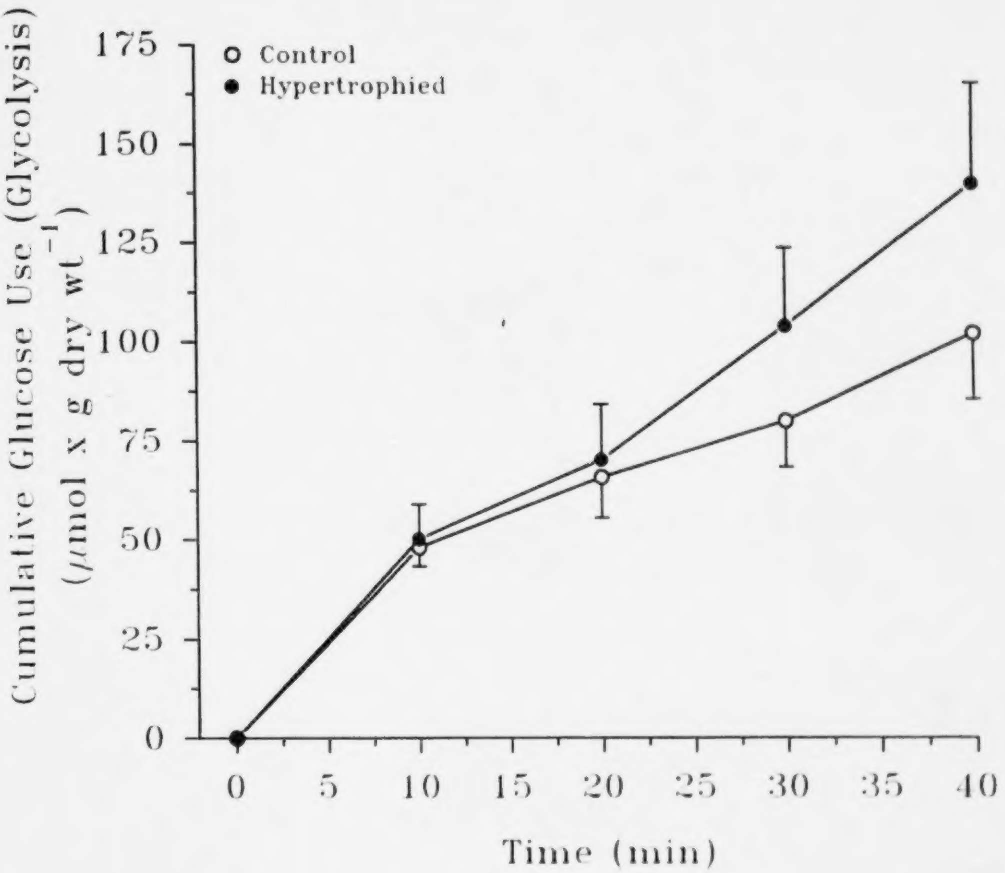

Figure 5-2: Cumulative glucose use (glycolysis) in control and hypertrophied rat hearts during 40 min of post-ischemic reperfusion. Cumulative rates of glycolysis were determined in hearts perfused with buffer containing 11 mM [5-^{3}H]glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ^3H_2O production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=8 (controls), n=6 (hypertrophied).

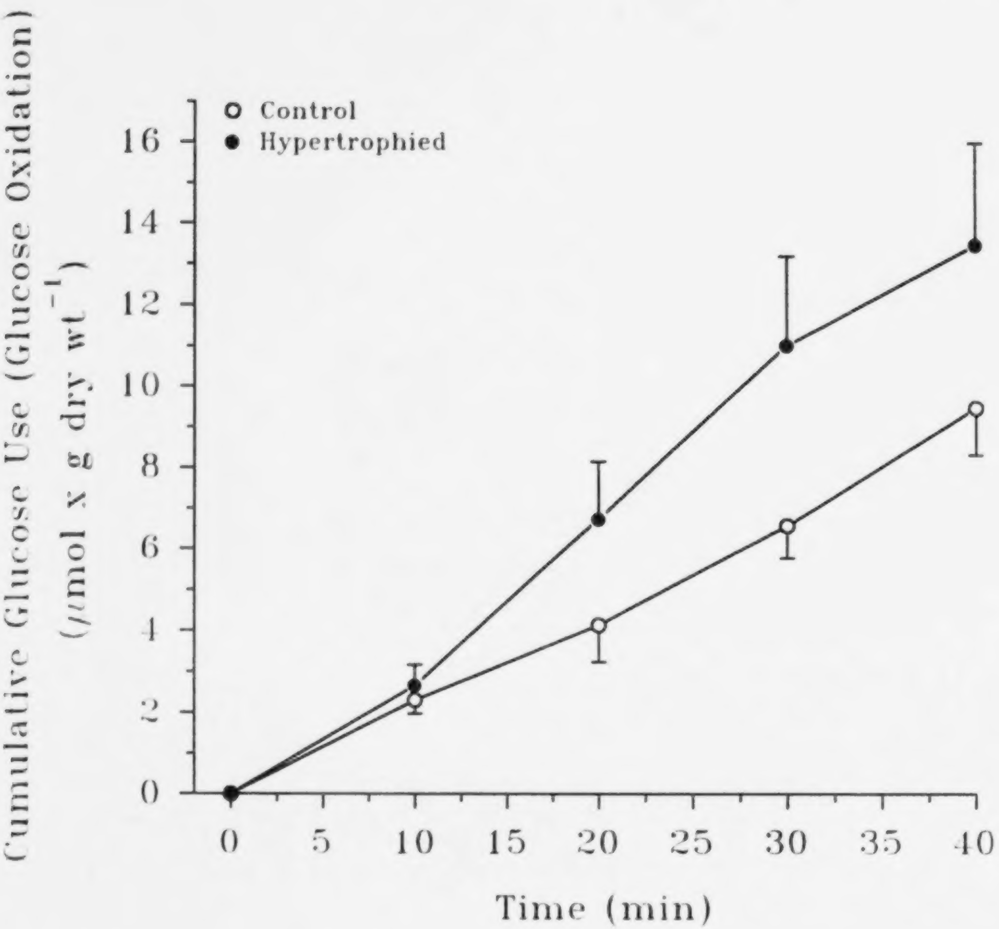

Figure 5-3: Cumulative glucose use (glucose oxidation) in control and hypertrophied rat hearts during 40 min of post-ischemic reperfusion. Cumulative rates of glucose oxidation were determined in hearts perfused with buffer containing 11 mM [U-^{14}C]glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. $^{14}CO_2$ production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=9 (controls), n=8 (hypertrophied).

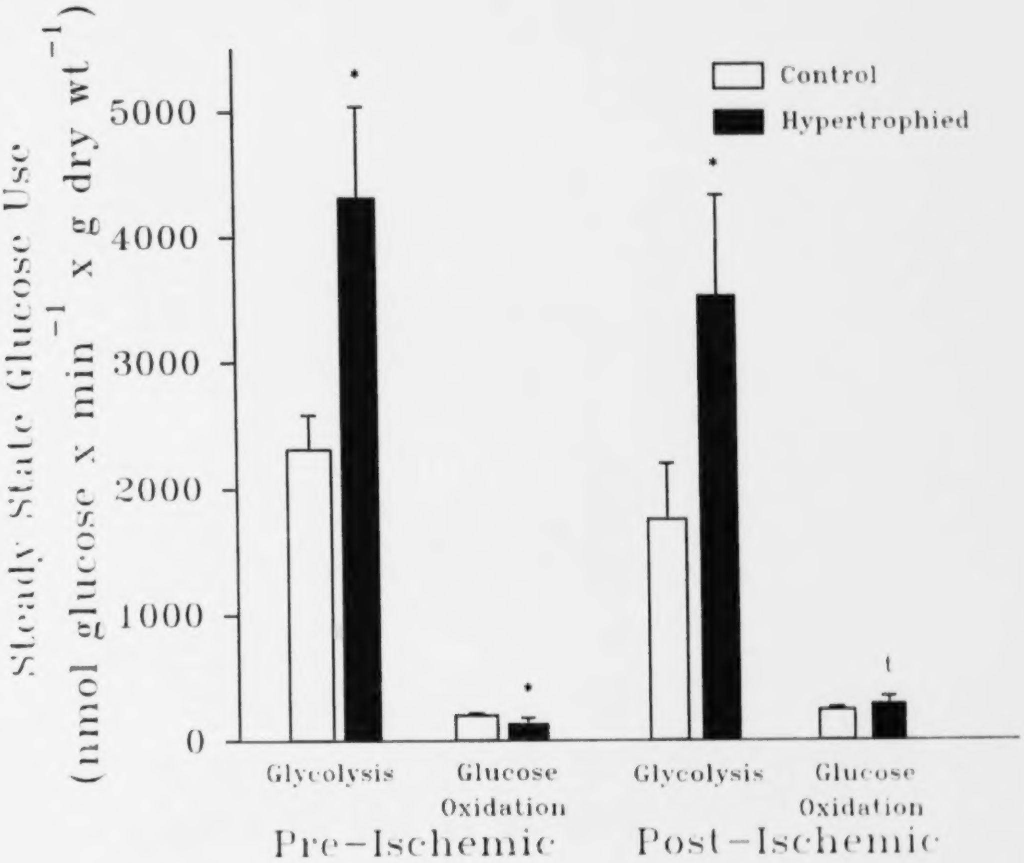

Figure 5-4: Steady state rates of glucose use (glycolysis and glucose oxidation) in control and hypertrophied rat hearts during pre-ischemic perfusion and post-ischemic reperfusion. Steady state rates of glycolysis and glucose oxidation were determined in hearts perfused with buffer containing 11 mM [5-^{3}H/U-^{14}C]glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ^3H_2O and $^{14}CO_2$ production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=8 (control glycolysis), n=6 (hypertrophied glycolysis), n=9 (control glucose oxidation), and n=8 (hypertrophied glucose oxidation). *significantly different from control hearts ($p \leq 0.05$). †significantly different from comparable pre-ischemic value ($p \leq 0.05$).

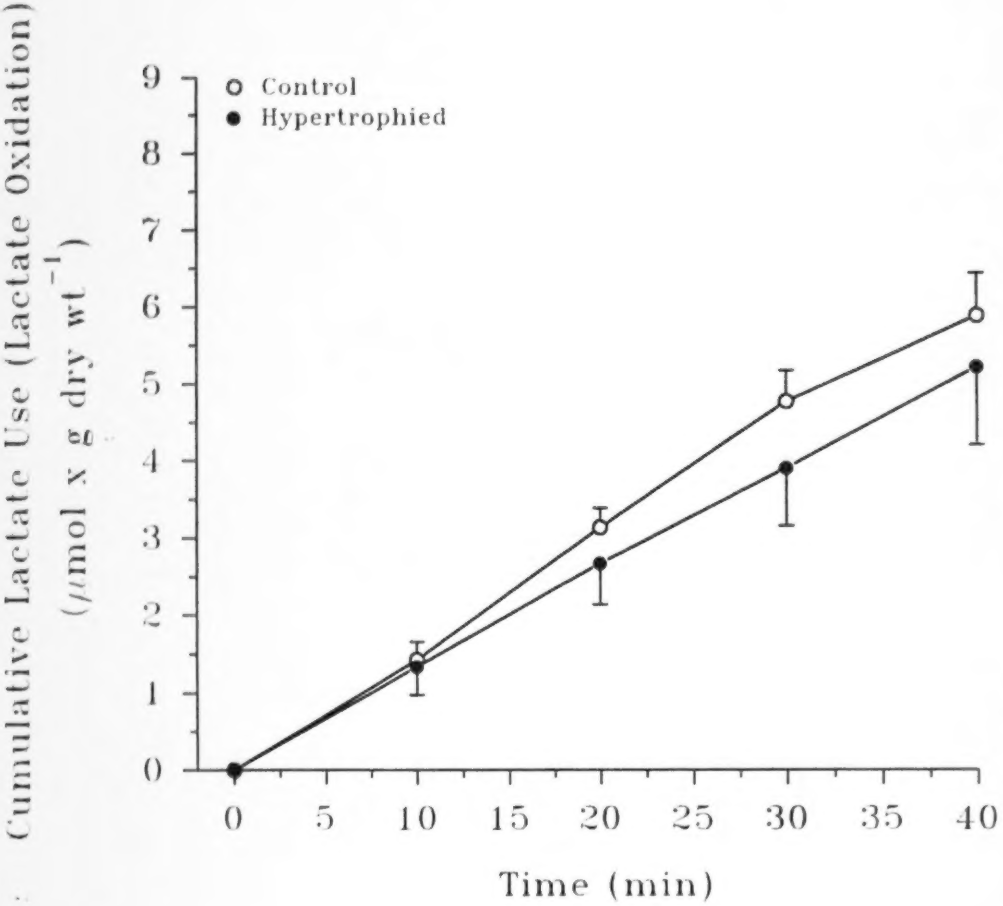

Figure 5-5: Cumulative lactate use (lactate oxidation) in control and hypertrophied rat hearts during 40 min of post-ischemic reperfusion. Cumulative rates of lactate oxidation were determined in hearts perfused with buffer containing 11 mM glucose, 0.5 mM [U-^{14}C]lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. $^{14}CO_2$ production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=5 (controls), n=8 (hypertrophied).

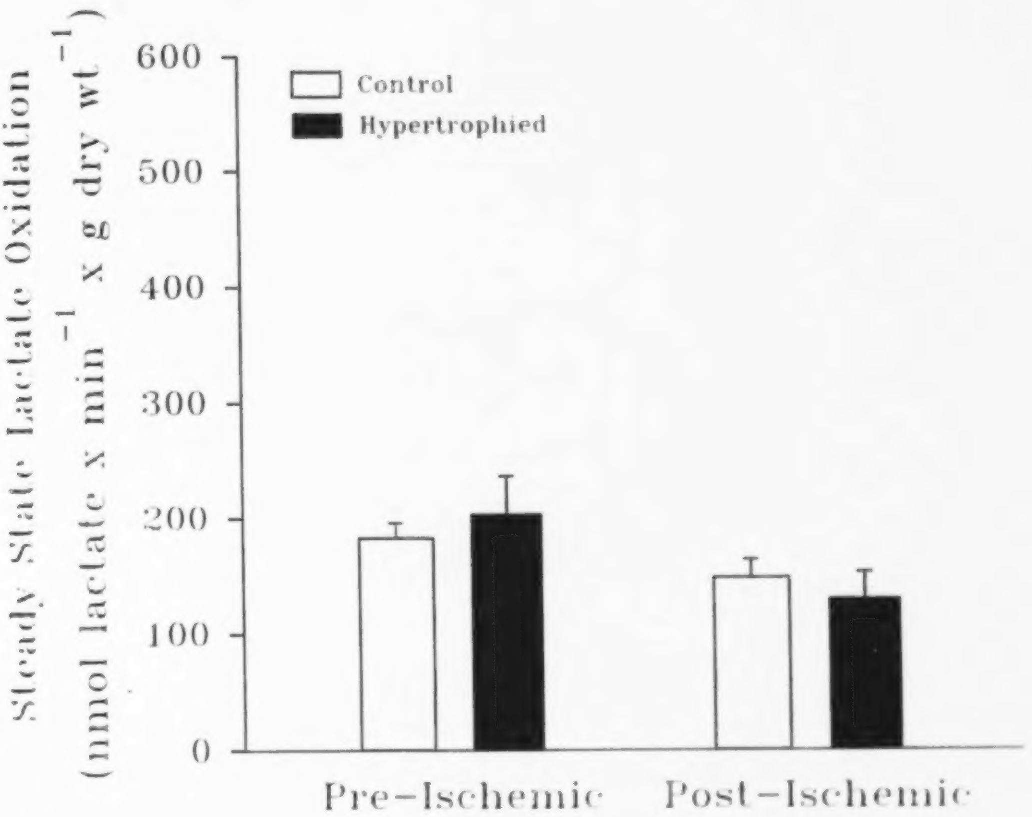

Figure 5-6: Steady state rates of lactate oxidation in control and hypertrophied rat hearts during pre-ischemic perfusion and post-ischemic reperfusion. Steady state rates of lactate oxidation were determined in hearts perfused with buffer containing 11 mM glucose, 0.5 mM [U-^{14}C]lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. $^{14}CO_2$ production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=5 (control), and n=8 (hypertrophied).

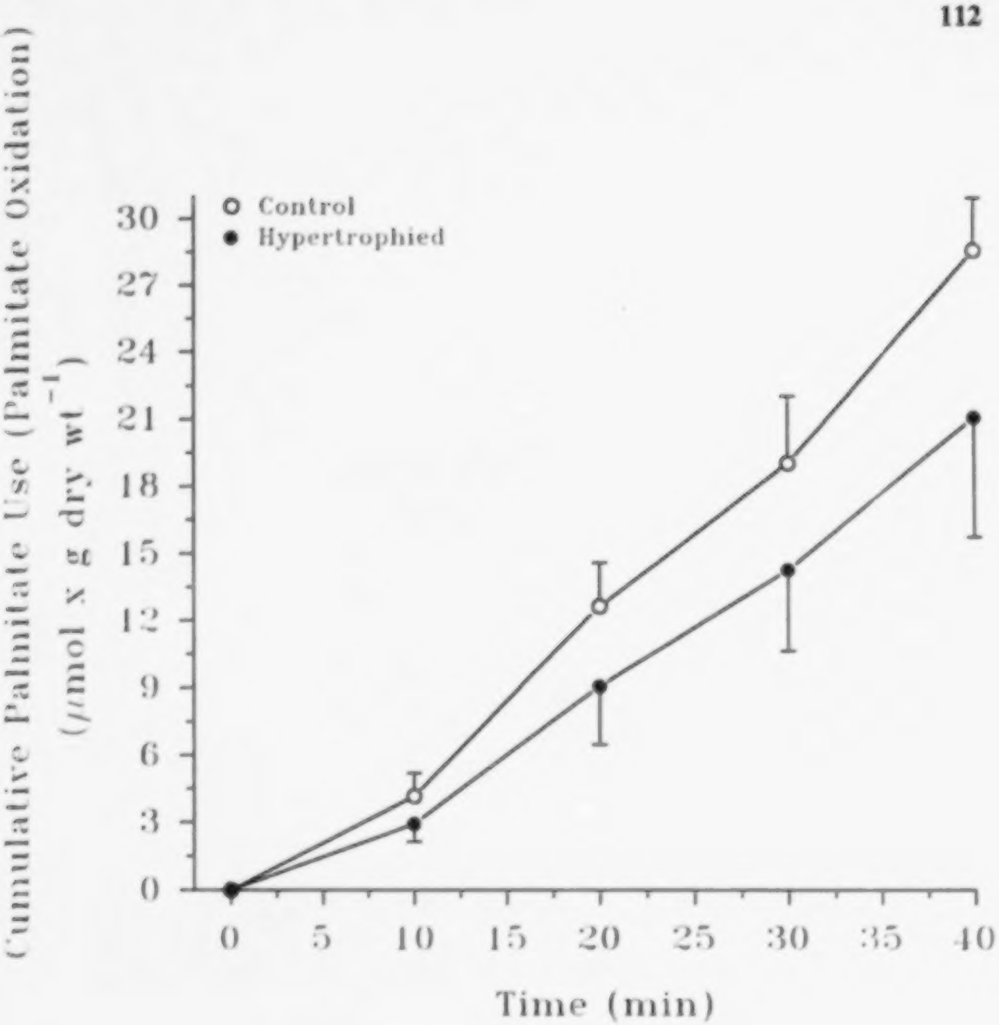

Figure 5-7: Cumulative palmitate use (palmitate oxidation) in control and hypertrophied rat hearts during 40 min of post-ischemic reperfusion. Cumulative rates of palmitate oxidation were determined in hearts perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM [9,10-^{3}H]palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ^3H_2O production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=5 (controls), n=8 (hypertrophied).

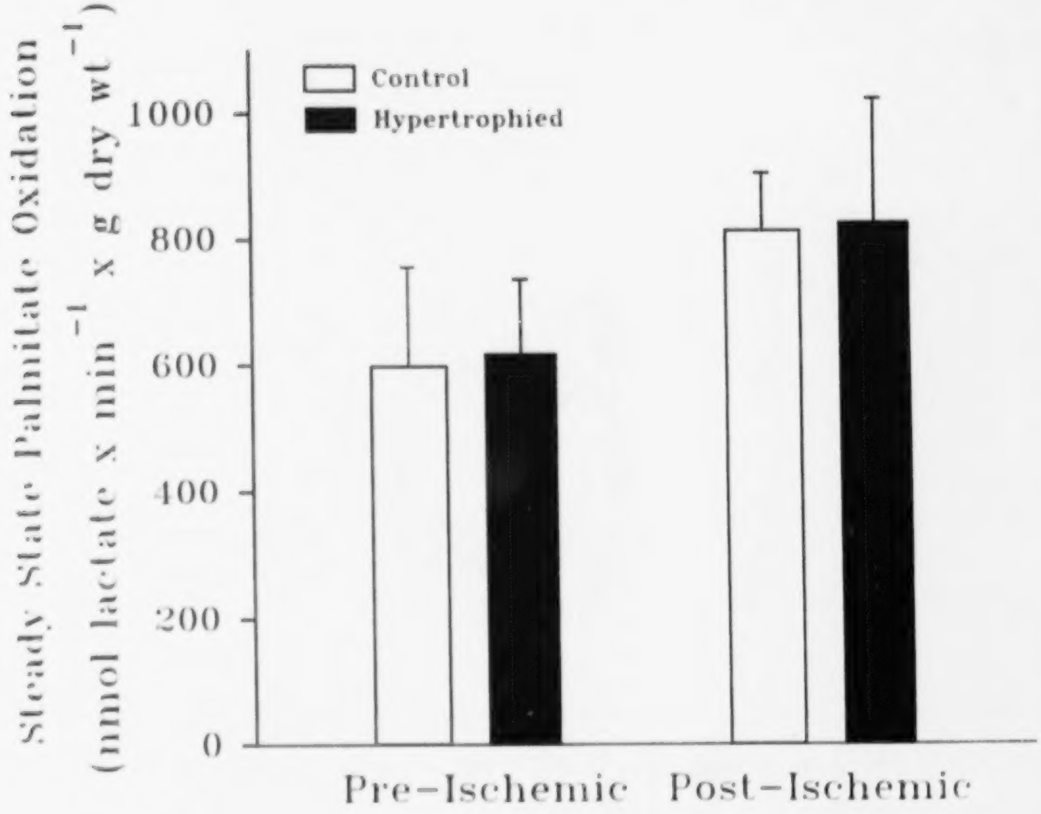

Figure 5-8: Steady state rates of palmitate oxidation in control and hypertrophied rat hearts during pre-ischemic perfusion and post-ischemic reperfusion. Steady state rates of palmitate oxidation were determined in hearts perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM [9,10-^{3}H]palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ^3H_2O production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=5 (control), and n=8 (hypertrophied).

Glycolysis:

$$\text{Glucose} + 2\ \text{ADP} + 2\ P_i \dashrightarrow 2\ \text{lactate} + 2\ \text{ATP} + 2\ H_2O$$

$$2\ \text{ATP} \dashrightarrow 2\ \text{ADP} + 2\ P_i + \mathit{2\ H^+}$$

Glycolysis and Glucose Oxidation:

$$\text{Glucose} + 38\ \text{ADP} + 38\ P_i + 6\ O_2 + \mathit{38\ H^+} \dashrightarrow 38\ \text{ATP} + 6\ CO_2 + 6\ H_2O$$

$$38\ \text{ATP} \dashrightarrow 38\ \text{ADP} + 38\ P_i + \mathit{38\ H^+}$$

Figure 5-9: Accountability of H^+ produced from glucose metabolism (glycolysis and glucose oxidation). Glycolysis is a net H^+ producing reaction upon the hydrolysis of ATP. Glycolysis coupled to Glucose oxidation is a H^+ neutral process when the ATP formed is hydrolysed.

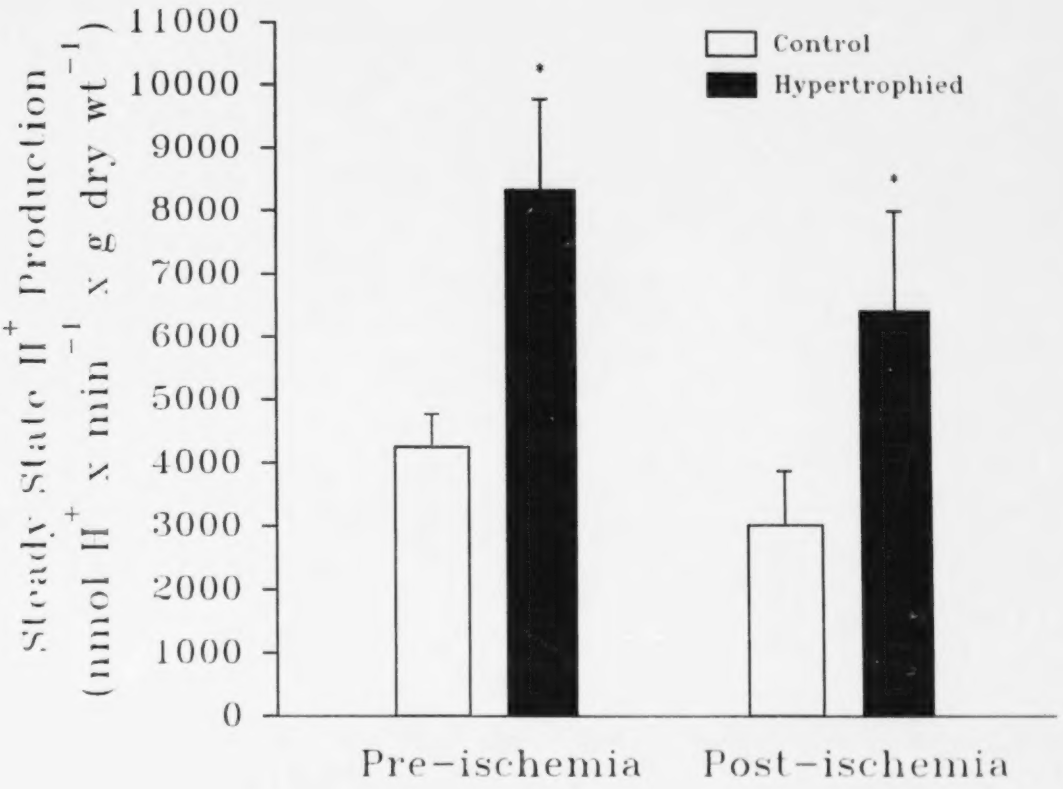

Figure 5-10: Steady state rates of H$^+$ production in control and hypertrophied rat hearts during pre-ischemic perfusion and post-ischemic reperfusion. Steady state rates of H$^+$ production were determined from rates of glycolysis and glucose oxidation (Figure 5-4) in hearts perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. H$^+$ production was calculated by subtraction of steady state glucose oxidation rates from steady state glycolysis and multiplied by a factor of 2 (see Figure 5-9). Values are the mean ± SEM, n=8 (control), and n=6 (hypertrophied). *significantly different from control hearts ($p \leq 0.05$).

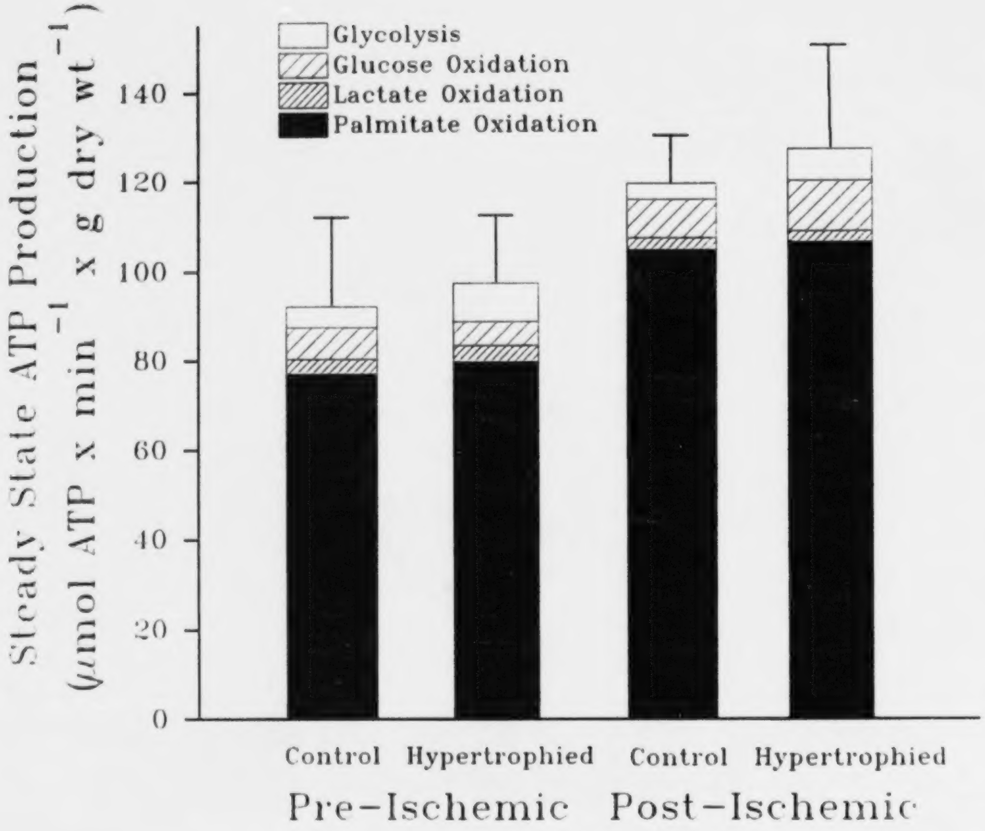

Figure 5-11: Steady state ATP production in control and hypertrophied rat hearts during pre-ischemic perfusion and post-ischemic reperfusion. Hearts were perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU$\cdot$ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ATP production rates were calculated from the data shown in Figures 5-4, 5-6, and 5-8 (calculations described in **CHAPTER II**).

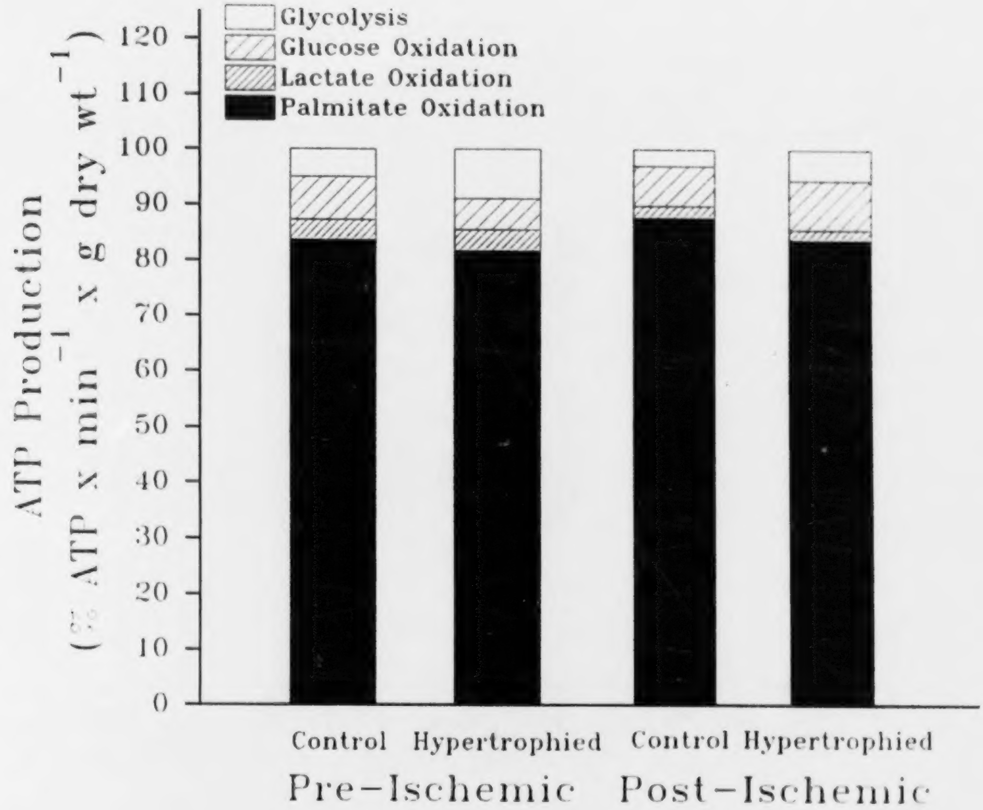

Figure 5-12: Percent ATP production in control and hypertrophied rat hearts during pre-ischemic perfusion and post-ischemic reperfusion. Rates of ATP production from glycolysis, glucose, lactate and palmitate oxidation (Figure 5-11) are expressed as percent contribution of total ATP production.

CHAPTER VI

Propionyl L-carnitine and its ability to increase glucose oxidation in the control and hypertrophied rat heart during aerobic perfusion[10]

A) Introduction

It is well established that tissue L-carnitine levels are decreased in the hypertrophied myocardium (Reibel et al., 1983; El Alaoui-Talibi et al., 1992; Motterlini et al., 1992; Yang et al., 1992; Michelletti et al., 1994ab; Cheikh et al., 1994). A limitation in the translocation of fatty acids into the mitochondria due to decreased L-carnitine levels has been suggested to lead to decreased ATP production from fatty acid oxidation, and therefore myocardial dysfunction (El Alaoui-Talibi et al., 1992; Cheikh et al., 1994). For this reason, restoring myocardial L-carnitine level is a potential therapeutic approach to ameliorate the pathophysiologic consequences of myocardial hypertrophy.

Propionyl L-carnitine (PLC) is a naturally occurring derivative of L-carnitine that has the potential to not only replenish myocardial L-carnitine stores (Paulson et al., 1986), but also to replenish key mitochondrial TCA cycle intermediates (Davies et al., 1980; Tassani et al., 1994). Although the beneficial effects of PLC have been suggested to occur via improvement of fatty acid oxidation, this has yet to be directly determined.

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[10] A version of this chapter has been published: Schönekess BO, Allard MF, and Lopaschuk GD. 1995. Circ Res 1995 77:726-734.

It has been demonstrated that increasing myocardial L-carnitine levels stimulates glucose oxidation in the normal heart (Broderick et al., 1992,1993). This stimulation of glucose oxidation can be attributed to the actions of L-carnitine on lowering intramitochondrial the acetyl-CoA/CoA ratio and relieving the inhibition of PDC (Lysiak et al., 1988; Broderick et al., 1992).

It is possible that the beneficial effects of PLC could also occur secondary to a stimulation of glucose oxidation. To date, the direct effects of PLC on overall energy substrate metabolism have not been determined during normal aerobic perfusion of working rat hearts. Nor has its effects been noted on glucose metabolism (glycolysis and glucose oxidation) during post-ischemic reperfusion. Therefore the effects of PLC on myocardial carbohydrate and fatty acid metabolism in hypertrophied rat hearts was determined. Rates of glycolysis, glucose oxidation, lactate oxidation and fatty acid oxidation were measured in isolated working hearts acutely pre-treated with PLC.

B) Methods

i) Hypertrophy model

Myocardial hypertrophy was produced in male Wistar-Kyoto rats as described in **CHAPTER II**.

ii) Specific perfusion protocol

For perfusions where the hearts were treated with PLC, 1 mM PLC (pH 7.4) was added to the initial 60 min period of working heart perfusions similar to that described in **CHAPTER V**. A 5 min Langendorff period is used to wash out PLC from the coronary circulation, and to allow for the replacement of the perfusion buffer with PLC free buffer. The hearts then went through 30 min of aerobic perfusion, a 30 min period of global ischemia, followed by 40 min of post-ischemic reperfusion.

C) Results

i) Heart weight & body weight of experimental animals

Heart and body weights of the experimental animals used for the acute PLC loading experiment were not different from the hearts used in the ischemia/reperfusion experiments (**CHAPTER V**, Table 5-1). At the end of the eight week study period, a mild cardiac hypertrophy was seen in the aortic banded rats, with heart weight increasing by 15% compared to sham-operated control rats. A significant hypertrophy was seen regardless of whetheror not the increase was based upon differences in wet heart weight or dry heart weight. Heart weight to body weight ratio was also significantly increased in the aortic-banded rats.

ii) Myocardial L-carnitine levels of control and hypertrophied rat hearts pretreated with 1 mM PLC

Total myocardial L-carnitine content in hypertrophied and control hearts compared to non PLC treated hearts, is shown in Figure 6-1. These hearts were frozen at the end of the 40 min post-ischemic reperfusion period. Similar to previous studies (Reibel et al., 1983; El-Alaoui-Talibi et al., 1992; Motterlini et al., 1992; Yang et al., 1992; Michelletti et al., 1994ab; Cheikh et al., 1994), a significant decrease in myocardial L-carnitine levels was seen in the hypertrophied hearts. Perfusion of hearts for a 60 min period with 1 mM PLC resulted in a significant increase in L-carnitine levels in both the control and hypertrophied hearts. Total myocardial L-carnitine content increased 35% in control hearts and 47% in hypertrophied hearts

iii) PLC effects on mechanical function in hypertrophied hearts

Cardiac work in hypertrophied and control hearts is shown in Figure 6-2. The mildly hypertrophied hearts used in this study showed no significant difference in cardiac work from control hearts at the initiation of the perfusion. The addition of 1 mM PLC did not initially alter cardiac work in either hypertrophied or control hearts. However, by 60 min of perfusion, a significant increase in cardiac work was observed in the PLC-treated hypertrophied hearts compared to untreated hypertrophied hearts. A similar increase was not observed in control hearts. The improvement in cardiac work in PLC-treated hypertrophied hearts persisted following the washout of PLC from the coronary circulation and replacement of the 1 mM PLC perfusate with PLC free perfusion buffer.

Table 6-1 shows the effects of PLC on other parameters of heart function at the end of the 30 min period following removal of PLC from the perfusate, and the addition of radiolabel. The main effect of PLC was to increase aortic output in the hypertrophied hearts, resulting in a significant increase in cardiac output. No major effect of PLC was observed on either HR, developed pressure, PSP or coronary flow.

It should be noted that although heart weight in the hypertrophied hearts was 15% higher than control hearts, coronary flow did not differ between the two groups. Despite this, it is unlikely that the hypertrophied hearts were perfused under slightly ischemic conditions. Mechanical function was well maintained in these hearts, and increased upon PLC supplementation without any further increase in coronary flow, suggesting that O_2 supply was not limited.

iv) PLC effects on glycolysis and glucose oxidation in control and hypertrophied hearts

Following removal of PLC, steady state rates of glycolysis and glucose oxidation were subsequently measured over the 30 min aerobic period (Figures 6-3). As shown in Figure 6-3, rates of glycolysis were significantly accelerated in hypertrophied hearts compared to control hearts. As expected, glucose oxidation rates were considerably lower than glycolytic rates in all hearts. This is due to the fact that fatty acids are more potent inhibitors of glucose oxidation than of glycolysis (secondary to inhibition of PDH by acetyl CoA derived from β-oxidation). Although glycolytic rates were elevated in hypertrophied hearts, a significant decrease in glucose oxidation rates were seen in these hearts compared to control hearts.

In hearts treated with PLC, there was a dramatic increase in glucose oxidation rates in both the control and hypertrophied heart (Figure 6-3). Rates of glucose oxidation increased (from 217 ± 26 to 858 ± 192 nmol·min^{-1}·g dry wt^{-1}) in control hearts and in hypertrophied hearts (from 137 ± 25 to 627 ± 110 nmol·min^{-1}·g dry wt^{-1}). The increase in glucose oxidation was also accompanied by a significant increase in glycolysis in control hearts (from 2320 ± 394 to 4183 ± 768 nmol·min^{-1}·g dry wt^{-1}). Rates of glycolysis were not further increased in hypertrophied hearts (3792 ± 352 *vs* 4467 ± 958 nmol·min^{-1}·g dry wt^{-1}).

v) PLC effects on lactate oxidation in control and hypertrophied hearts

Rates of lactate oxidation in hypertrophied and control hearts are shown in Figure 6-4. Similar to glucose oxidation rates, lactate oxidation rates were significantly depressed in the hypertrophied hearts compared to control hearts (119 ± 17 *vs* 194 ± 25 nmol·min^{-1}·g dry wt^{-1}, respectively). PLC treatment significantly increased the rate of lactate oxidation in hypertrophied hearts (from 119 ± 17 to 252 ± 47 nmol·min^{-1}·g dry wt^{-1}). In contrast, lactate oxidation rates were not significantly increased in control hearts treated with PLC (from 194 ± 25 to 254 ± 62 nmol·min^{-1}·g dry wt^{-1}).

vi) PLC effects on palmitate oxidation in control and hypertrophied hearts

Rates of palmitate oxidation in hypertrophied and control hearts are shown in Figure 6-4. No differences in steady state rates of palmitate oxidation were seen in either group. This differs from hearts perfused with low concentrations of palmitate (0.4 mM) in which we previously observed a significant decrease in fatty acid oxidation in hypertrophied hearts compared to control hearts (Figure 3-3). PLC treatment significantly increased palmitate oxidation in hypertrophied hearts (from 380 ± 32 to 530 ± 57 $\mathrm{nmol \cdot min^{-1} \cdot g\ dry\ wt^{-1}}$) but not in PLC-treated control hearts (429 ± 59 *vs* 395 ± 35 $\mathrm{nmol \cdot min^{-1} \cdot g\ dry\ wt^{-1}}$).

vii) Steady State Rates of ATP Production in hypertrophied hearts

Overall rates of ATP produced from exogenous carbon substrates were calculated from the steady state glycolytic and oxidative rates obtained from Figures 6-3 to 6-5. Absolute rates of ATP production in control and hypertrophied hearts are shown Figure 6-6. Similar rates of overall ATP production were observed in control and hypertrophied rat hearts perfused in the absence of PLC. PLC treatment increased rates of ATP production in both control and hypertrophied hearts. This increase was mainly due to effects of PLC on carbohydrate oxidation.

Figure 6-7 shows the percent contribution of glycolysis, glucose oxidation, lactate oxidation and fatty acid oxidation to ATP production. The most significant finding was that, in the absence of PLC, control hearts derived 7% of ATP production from glycolysis, compared to 12% in hypertrophied hearts. Conversely, the contribution of palmitate oxidation to ATP production was lower in the hypertrophied hearts (77%) compared to control hearts (80%). In PLC treated hearts, a dramatic increase in the relative contribution of glucose oxidation to ATP production occurred in both hypertrophied hearts (from 8 to 22%) and control hearts (from 8 to 33%). The relative contribution of lactate to ATP production did not change dramatically in any of the experimental groups. In the PLC-treated hypertrophied hearts, the increase in absolute rates of palmitate oxidation seen in Figure 6-5 did not translate into a relative increase in palmitate oxidation to ATP production. This occurred due to the increase in palmitate oxidation being paralleled by the increase in mechanical function and overall ATP production seen in these hearts (Figure 6-5).

viii) Effects of PLC on glycolysis and oxidative metabolism during post-ischemic reperfusion

Figure 6-8 shows the effects of PLC pre-treatment in control and hypertrophied hearts on post-ischemic rates of glycolysis. Glycolytic rates in control hearts significantly increased in the PLC treated group, and drop slightly in the hypertrophied hearts. PLC effectively increased rates of glucose oxidation during the post-ischemic reperfusion period in both the control and hypertrophied hearts. This effect on glucose oxidation was similar to what had previously been shown in normal hearts treated with

L-carnitine (Broderick et al., 1993). When rates of H^+ production are calculated during the post-ischemic reperfusion period, the untreated hypertrophied heart had a greater rate of H^+ production than control hearts ($p \leq 0.05$).

Although an increase in glucose oxidation occurred in both control and hypertrophied hearts, there was no significant improvement in mechanical function, measured as HR·PSP. Hypertrophied hearts showed a mechanical function of 12.67 ± 2.81 beats·mm Hg·10^{-3} (n=6) at the end of post-ischemic reperfusion when pre-treated with 1 mM PLC. This compared to a mechanical function of 10.31 ± 2.16 beats·mm Hg·10^{-3} (n=8) when not treated with PLC. Control hearts actually showed a depression in mechanical function at the end of post-ischemic reperfusion, going from 18.66 ± 1.67 (n=15) to 8.87 ± 2.53 (n=6) beats·mm Hg·10^{-3} in 1 mM PLC pre-treated hearts.

D) Summary

The findings of this chapter show that;

1) acute PLC treatment will increase carbohydrate oxidation during normal aerobic perfusion of control and hypertrophied hearts,

2) there is an increase in the mechanical function of the hypertrophied heart compared to control heart when the hearts are pretreated with 1 mM PLC,

3) upon post-ischemic reperfusion an increase in the rates of glucose oxidation in both control and hypertrophied hearts occurs,

4) however there is no significant improvement in the mechanical function of either

control or hypertrophied hearts pretreated with PLC during post-ischemic reperfusion,

5) an increase in H^+ ion production in control hearts and the accelerated pre-ischemic rates of H^+ production that exist in hypertrophied hearts may be the cause of the depressed post-ischemic functional recovery.

Table 6-1: Functional parameters of control and hypertrophied hearts during aerobic perfusion with and without 1 mM PLC pre-treatment

Parameter Measured	No PLC Control (n=15)	No PLC Hypertrophied (n=15)	1 mM PLC Loaded Control (n=15)	1 mM PLC Loaded Hypertrophied (n=15)
Heart Rate (beats·min^{-1})	220 ± 11	224 ± 12	239 ± 14	246 ± 14
Peak Systolic Pressure (mm Hg)	121 ± 4	120 ± 3	115 ± 4	121 ± 4
Developed Pressure (mm Hg)	50 ± 5	48 ± 7	47 ± 6	55 ± 6
Cardiac Output (ml·min^{-1})	44 ± 4	42 ± 3	48 ± 5	58 ± 4[†]
Aortic Output (ml·min^{-1})	16 ± 3	16 ± 3	23 ± 3	29 ± 3[†]
Coronary Flow (ml·min^{-1})	28 ± 3	27 ± 3	26 ± 3	29 ± 3

[*] significantly different from contol value ($p \leq 0.05$).

[†] significantly different from comparable no LPC value ($p \leq 0.05$).

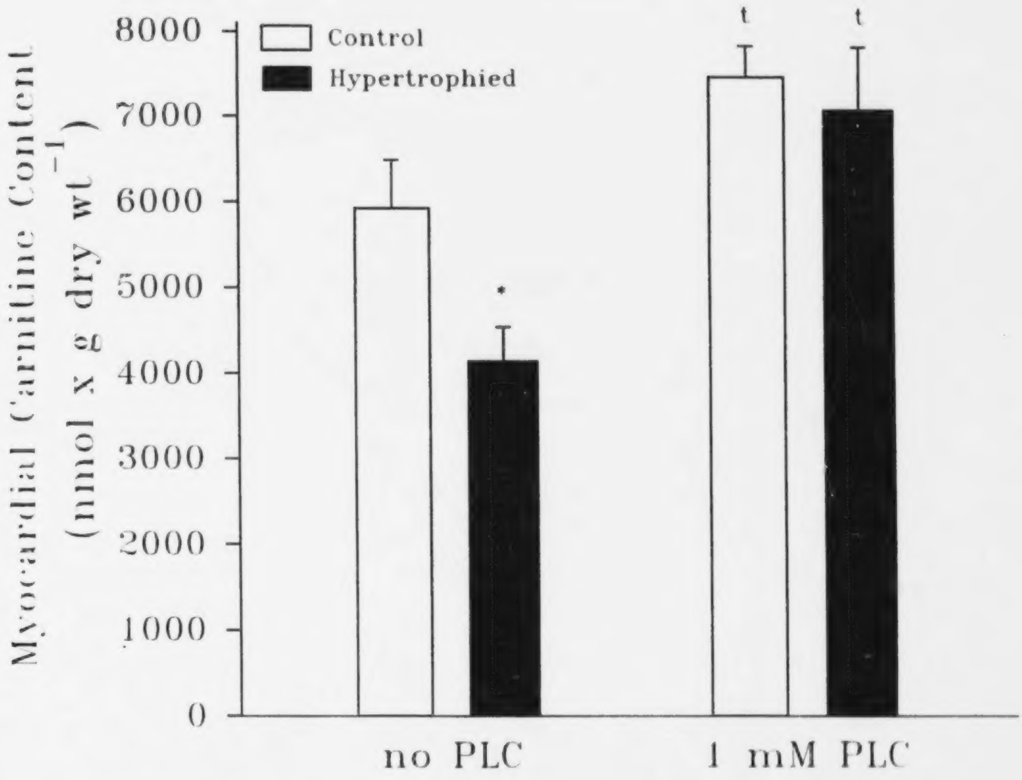

Figure 6-1: Myocardial carnitine content in control and hypertrophied rat hearts with and without 1 mM PLC loading. Hearts were perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg, for 60 min with or without 1 mM PLC. After the initial 60 min pulse, the hearts were switched to a 5 min Langendorff perfusion in order to change buffer to exclude PLC, and substrate metabolism was measured (described later). Total myocardial carnitine was measured as described in **CHAPTER II.** Values are the mean ± SEM, n=7 (untreated control), n=5 (untreated hypertrophied), n=6 (1 mM PLC treated control), and n=6 (1 mM PLC treated hypertrophied). *significantly different from control hearts ($p \leq 0.05$). ^{t}significantly different from comparable no PLC value ($p \leq 0.05$).

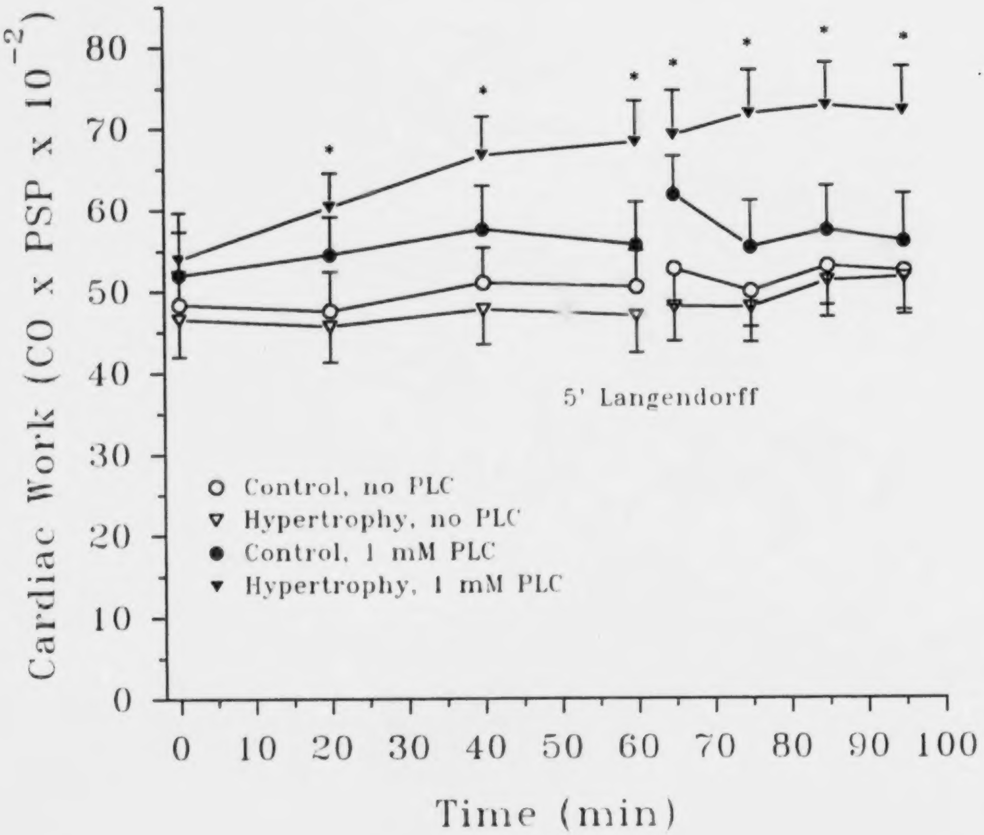

Figure 6-2: Mechanical function of control and hypertrophied rat hearts with and without 1 mM PLC loading. Mechanical function is expressed as cardiac work ($CO \cdot PSP \cdot 10^{-2}$). Hearts were perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and $100 \mu U \cdot ml^{-1}$ insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. Values are the mean ± SEM, n=15 (untreated control), n=15 (untreated hypertrophied), n=13 (1 mM PLC treated control), and n=16 (1 mM PLC treated hypertrophied). *significantly different from untreated hypertrophied hearts ($p \leq 0.05$)

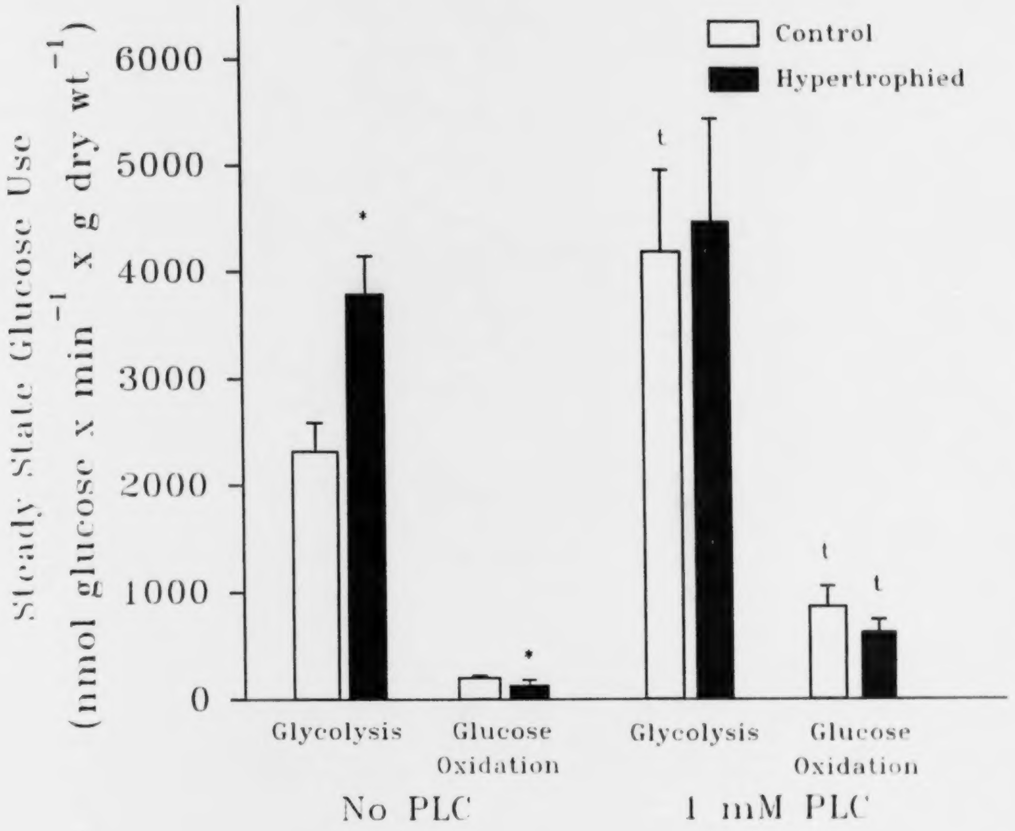

Figure 6-3: Steady state rates of glucose use (glycolysis and glucose oxidation) in control and hypertrophied rat hearts with and without 1 mM PLC loading. Steady state rates of glycolysis and glucose oxidation were determined in hearts perfused with buffer containing 11 mM [5-^{3}H/^{14}C]glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ^3H_2O and $^{14}CO_2$ production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=8 (untreated control), n=6-8 (untreated hypertrophied), n=6 (1 mM PLC treated control), and n=6 (1 mM PLC treated hypertrophied). *significantly different from control hearts ($p \leq 0.05$). ^{t}significantly different from comparable untreated value ($p \leq 0.05$).

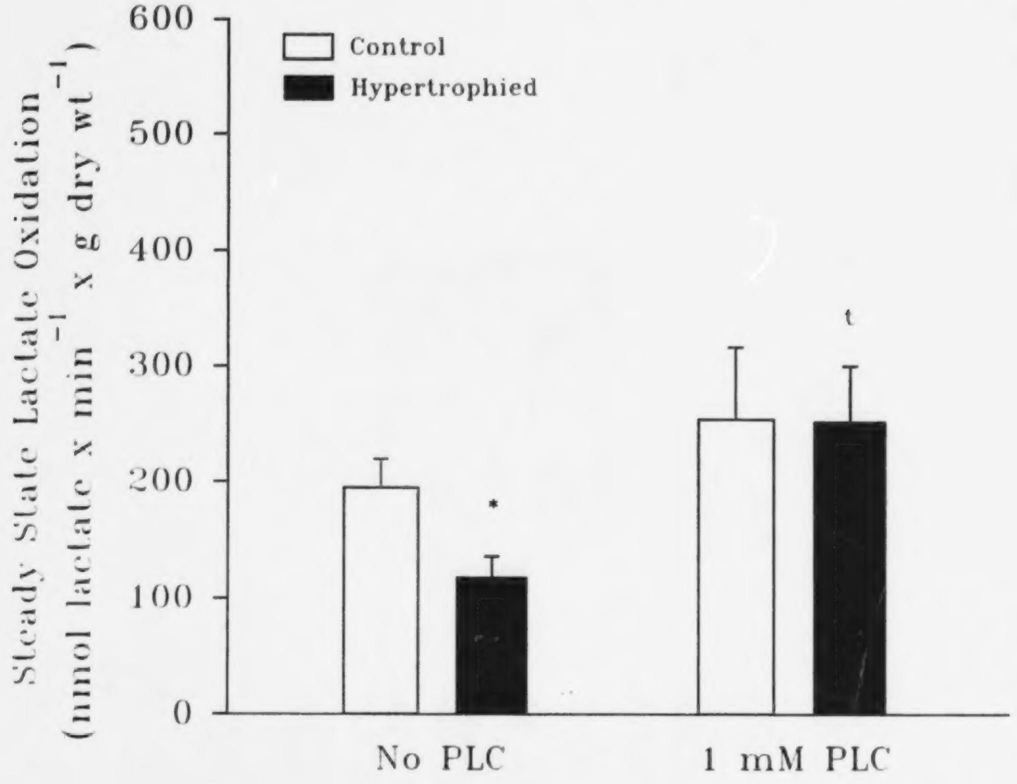

Figure 6-4: Steady state rates of lactate oxidation in control and hypertrophied rat hearts with and without 1 mM PLC loading. Steady state rates of lactate oxidation were determined in hearts perfused with buffer containing 11 mM glucose, 0.5 mM [U-^{14}C]lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. $^{14}CO_2$ production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=6 (untreated control), n=6 (untreated hypertrophied), n=7 (1 mM PLC treated control), and n=10 (1 mM PLC treated hypertrophied). *significantly different from control hearts ($p \leq 0.05$). ^{t}significantly different from comparable untreated value ($p \leq 0.05$).

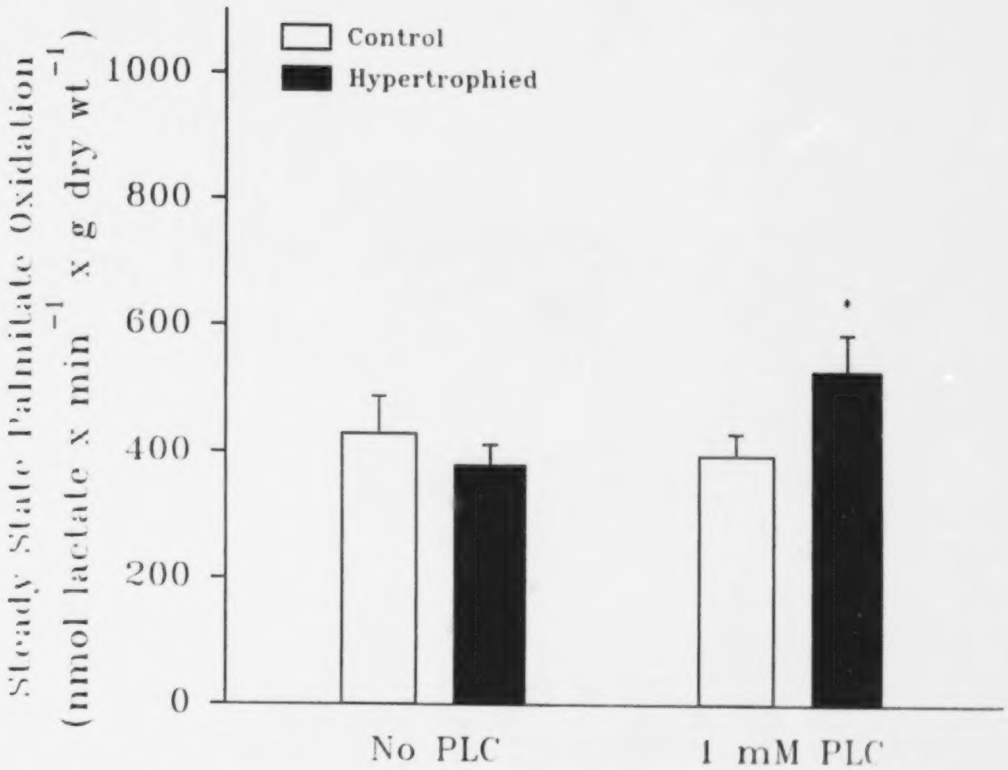

Figure 6-5: Steady state rates of palmitate oxidation in control and hypertrophied rat hearts with and without 1 mM PLC loading. Steady state rates of palmitate oxidation were determined in hearts perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM [9,10-^{3}H]palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ^3H_2O production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=6 (untreated control), n=7 (untreated hypertrophied), n=6 (1 mM PLC treated control), and n=10 (1 mM PLC treated hypertrophied). *significantly different from comparable untreated value ($p \leq 0.05$).

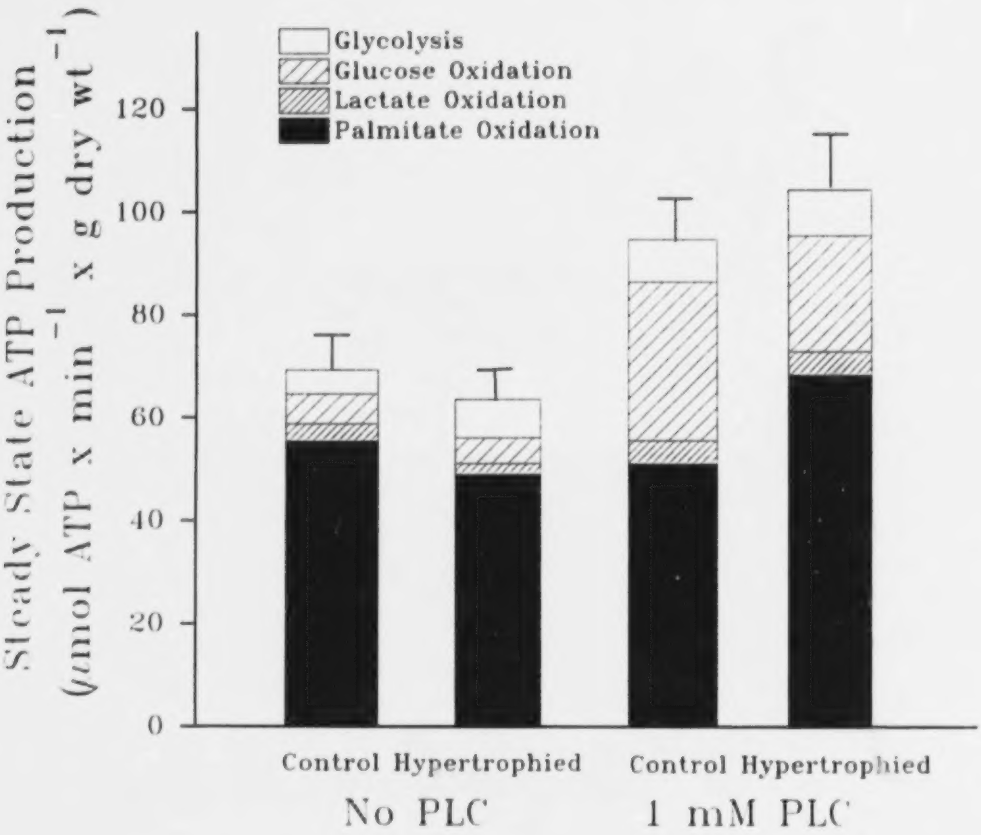

Figure 6-6: Steady state ATP production in control and hypertrophied rat hearts with and without 1 mM PLC loading. Hearts were perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and $100\mu U \cdot ml^{-1}$ insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ATP production rates were calculated from the data shown in Figures 6-3 to 6-5 (calculations described in **CHAPTER II**).

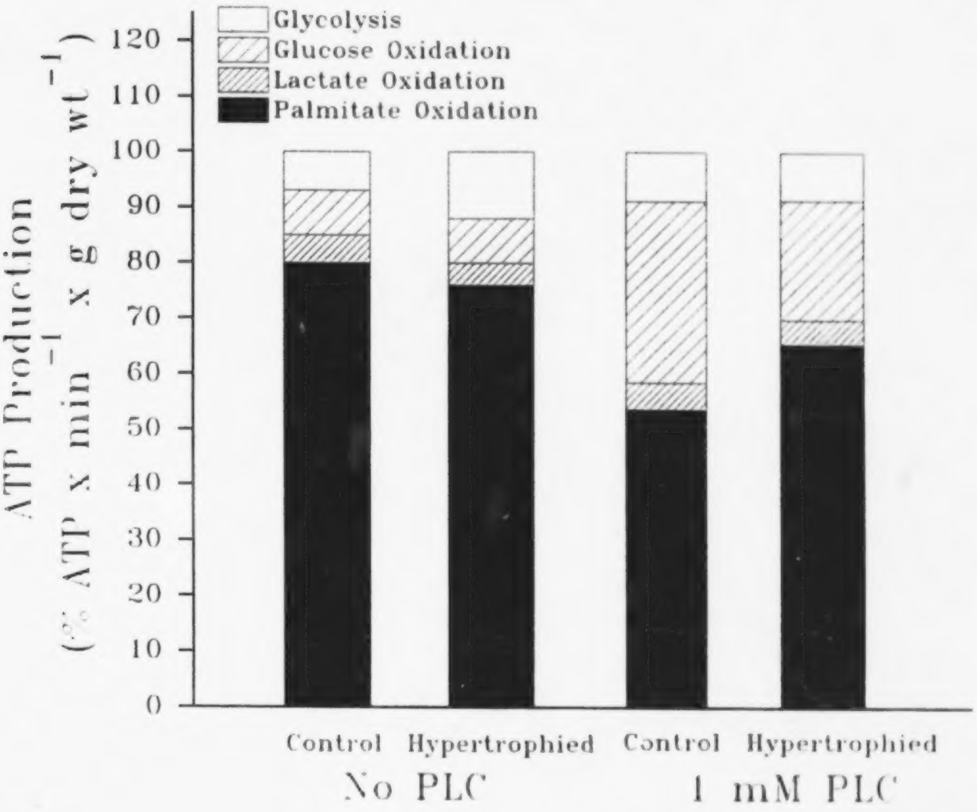

Figure 6-7: Percent ATP production in control and hypertrophied rat hearts with and without 1 mM PLC loading. Rates of ATP production from glycolysis, glucose, lactate and palmitate oxidation (Figure 6-6) are expressed as percent contribution of total ATP production.

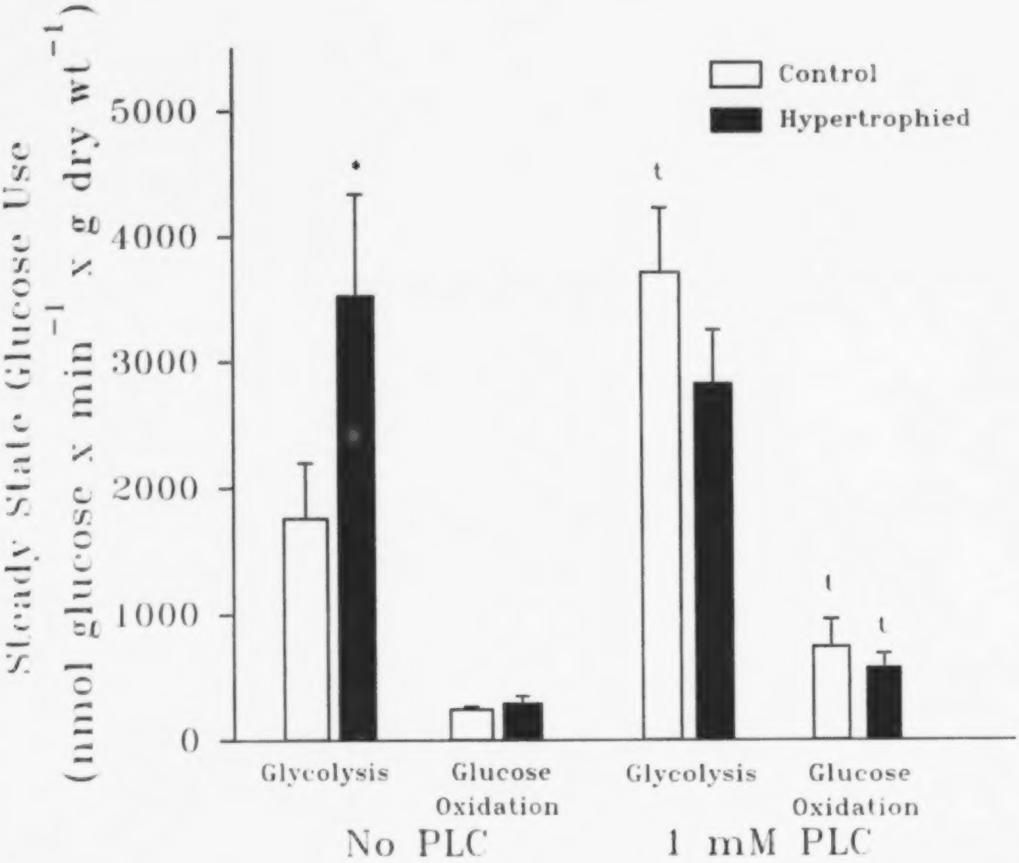

Figure 6-8: Steady state rates of glucose use (glycolysis and glucose oxidation) during post-ischemic reperfusion in control and hypertrophied rat hearts with and without 1 mM PLC loading. Steady state rates of glycolysis and glucose oxidation were determined in hearts perfused with buffer containing 11 mM [5-^{3}H/^{14}C]glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. ^3H_2O and $^{14}CO_2$ production were measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=8 (untreated control), n=6-8 (untreated hypertrophied), n=6 (1 mM PLC treated control), and n=6 (1 mM PLC treated hypertrophied). [*]significantly different from control hearts ($p \leq 0.05$). [t]significantly different from comparable untreated value ($p \leq 0.05$).

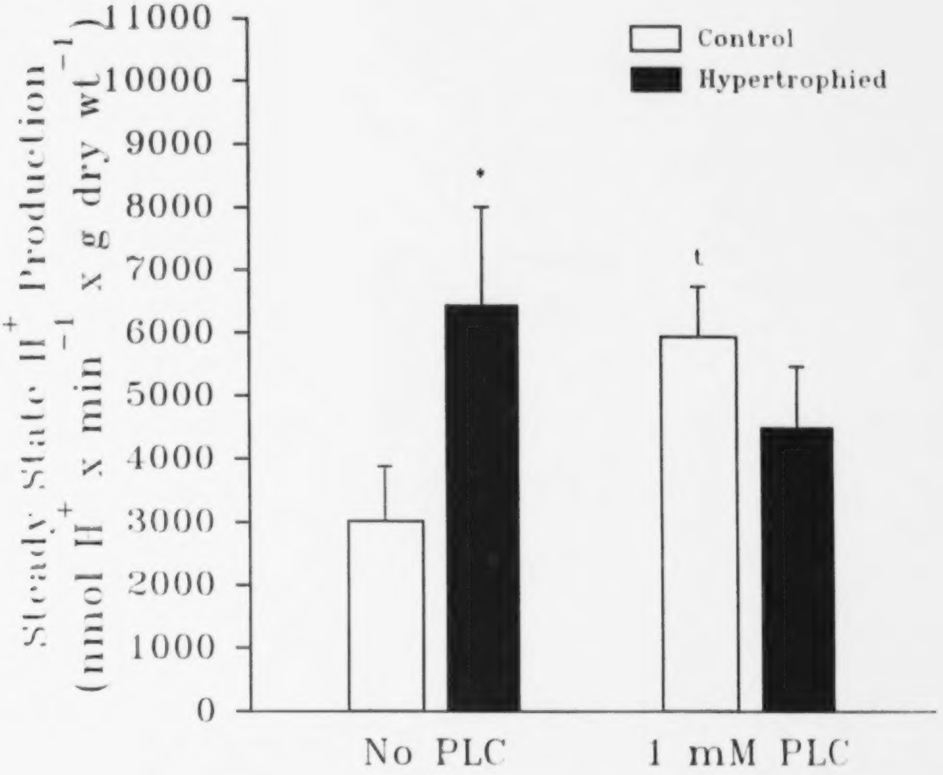

Figure 6-9: Steady state rates of H$^+$ production during post-ischemic reperfusion in control and hypertrophied rat hearts with and without 1 mM PLC loading. Steady state rates of H$^+$ production were determined from rates of glycolysis and glucose oxidation (Figure 6-7) in hearts perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. H$^+$ production was calculated by subtraction of steady state glucose oxidation rates from steady state glycolysis and multiplied by a factor of 2 (see Figure 5-9). Values are the mean ± SEM, n=8 (untreated control), n=6 (untreated hypertrophied), n=6 (1 mM PLC treated control), and n=6 (1 mM PLC treated hypertrophied). *significantly different from control hearts ($p \leq 0.05$). ^{t}significantly different from comparable untreated value ($p \leq 0.05$).

CHAPTER VII

Contribution of glycogen and exogenous glucose to carbohydrate metabolism during low-flow ischemia in control and hypertrophied rat hearts[11]

A) Introduction

As this thesis has shown, a pressure-overload myocardial hypertrophy is associated with an altered energy substrate usage. This is supported by additional studies by Bishop and Aultschuld (1971), Kagaya et al. (1990), El Alaoui-Talibi et al. (1992), and Cheikh et al. (1994). The key metabolic alteration that exists in the hypertrophied heart is increased glycolytic rates (Figure 3-1 and Figure 5-6) and an increased production of H^+ due to increased rates of glycolysis uncoupled from glucose oxidation (Figure 5-10).

During hypoxia or ischemia, oxidative metabolism is severely depressed, and glycolysis becomes a more important source of ATP production with glycogen breakdown contributing as a source of glucose for glycolysis. During low-flow ischemia, there may exist some residual oxidative metabolism from exogenous sources such as fatty acids, glucose and lactate, but the loss of O_2 supply due to the limited flow will severely limit oxidative metabolism (Neely and Morgan, 1974).

The exact contribution of glycogen to glycolysis during a low-flow ischemia has not been established. Neither is it definitively known if the glycolytic pathway is inhibited or accelerated in the hypertrophied heart, as has been suggested by a number of studies of ischemia (Anderson et al., 1990a; Cunningham et al., 1990; Allard et al., 1994b). Since glycolytic rates are accelerated in the hypertrophied heart during aerobic perfusion (Figure 3-1 and Figure 5-6) and there is evidence of an altered glycolytic isoenzyme content in the hypertrophied heart (Bishop and Aultschuld, 1971; Ingwall 1984), it is reasonable to suggest that during low-flow ischemia glycolytic rates will also be accelerated. However it is not known for certain if this does indeed occur. There may exist a point during ischemia at which glycolysis is maximal due to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity becoming rate limiting (Taegtmeyer, 1985). If this is the case, then the hypertrophied heart may not have an altered glucose metabolism during ischemia even though several enzymes in the glycolytic pathway are in greater abundance or have an altered activity (Bishop and Aultschuld, 1971; Ingwall 1984).

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[11] A version of this chapter has been submitted for publication. Schönekess BO, Allard MF, Henning SL, Wambolt RB, and Lopaschuk GD. 1995. Am J Physiol.

To ascertain the contribution of glycogen and exogenous glucose to glycolysis and residual glucose oxidation, a protocol was devised to radiolabel the glycogen pools of normal and hypertrophied rat hearts *in vitro*, and then follow the metabolism of the glycogen pool. This allowed for the determination of glycolysis and glucose oxidation rates from glycogen and exogenous glucose in the hypertrophied heart during a low-flow ischemia.

B) Methods

i) Hypertrophy model

Myocardial hypertrophy was produced in male Sprague-Dawley rats as described in **CHAPTER II**.

ii) Specific perfusion protocol (Figure 7-1)

Following cannulation of the aorta and left atria, the hearts were perfused for 30 min in the Langendorff mode with a Krebs'-Henseleit buffer containing 2.50 mM Ca^{2+}, with no exogenous carbon substrates (Figure 7-1). Perfusion pressure was maintained at 60 mm Hg throughout this depletion protocol. A series of hearts were frozen at the end of the 30-min depletion period to measure tissue glycogen, lactate, and high energy phosphates (not shown in Figure 7-1).

After the depletion protocol, the hearts were subsequently switched to a working mode, which allowed for left ventricular filling via the left atria. The perfusate was a Krebs'-Henseleit buffer which now contained 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate prebound to 3% bovine serum albumin, and 100 $\mu U \cdot ml^{-1}$ insulin. Perfusion pressures of 11.5 mm Hg left atrial preload and 80 mm Hg aortic afterload were used. Hearts were perfused for 60 min to resynthesize the glycogen pool.

To label the glycogen pool during the resynthesis period, a [5-^{3}H]glucose label was initially added to the buffer at the start of the working heart mode (Series A). With the [5-^{3}H]glucose label present during the resynthesis period, aerobic rates of glycolysis from exogenous glucose were measured. Series B hearts were frozen here to measure tissue glycogen, lactate, high energy phosphates, and the percent of radiolabelling of the glycogen pool. During low-flow ischemia the presence of the [5-^{3}H]glucose label in the perfusate allowed for the determination of total rates of glycolysis from endogenous glycogen ([5-^{3}H]glucose labelled glycogen) and exogenous glucose. A parallel series of hearts had the glycogen pool labelled with [U-^{14}C]glucose during the resynthesis period (Series C). This allowed for the measurement of aerobic rates of glucose oxidation during the resynthesis period, and total rates of glucose oxidation from endogenous glycogen ([U-^{14}C]glucose labelled glycogen) and exogenous glucose during low-flow ischemia. Using this protocol, the glycogen pool at the end of the resynthesis period was labelled with either [5-^{3}H]glucose (Series A) or [U-^{14}C]glucose (Series B).

At the end of the resynthesis period, hearts were subjected to a low-flow ischemia similar to what has previously been used (Lopaschuk et al., 1992; Schönekess et al., 1995c). Upon switching the hearts to low-flow ischemia (initiated by an aortic retrograde perfusion of 0.5 ml·min^{-1}) the other radiolabel of glucose was added to the perfusion system so that dual labelling now occurred; i.e., Series A: [5-^{3}H]glucose during resynthesis followed by [U-^{14}C]glucose added at the start of low-flow, and Series C: [U-^{14}C]glucose during resynthesis followed by [5-^{3}H]glucose added at the start of low-flow. Series A hearts ([5-^{3}H]glucose followed by [U-^{14}C]glucose) allows the additional measurement of the contribution of exogenous glucose to glucose oxidation during low-flow ischemia. Whereas, Series C hearts ([U-^{14}C]glucose followed by [5-^{3}H]glucose) allow for the additional measurement of glycolysis from exogenous glucose during low-flow ischemia.

To calculate the contribution of glycogen to glycolysis during low-flow ischemia, rates of glycolysis from exogenous glucose (Series C) were subtracted from total rates of glycolysis taken during low-flow ischemia (Series A). Calculation of glucose oxidation from endogenous glycogen during low-flow ischemia was achieved in a similar manner; namely, rates of glucose oxidation from exogenous glucose (Series A) were subtracted from total rates of glucose oxidation during low-flow ischemia (Series C).

Perfusate buffer samples required for metabolic analysis were taken at 10, 20, 40, and 60 min during the resynthesis period. During low-flow ischemia, samples of perfusion buffer were taken at 5, 10, 20, 40, and 60 min. Hearts were frozen at the end of the glycogen depletion period, at the end of the resynthesis period (Series B) and at the end of low-flow ischemia (Series A, and C) for the measurement of glycogen, lactate and high energy phosphate content.

C) Results

i) Heart weight & body weight of experimental animals

Table 7-1 contains the body and wet heart weights of control and aortic banded animals (hypertrophied hearts) eight weeks after the banding procedure was performed.

There was a significant increase in heart weight of hypertrophied hearts compared to control hearts (21%). This increase in heart weight was not accompanied by an increase in body weight after eight weeks. Therefore, aortic-banded animals also had a significantly increased heart weight to body weight ratio compared to control animals (21%).

ii) Myocardial function of control and hypertrophied rat hearts during the aerobic glycogen resynthesis period

Functional measurements were taken throughout the glycogen resynthesis period. Recovery of function after the 30 min depletion period was immediate (Table 7-2). Control and hypertrophied hearts had similar mechanical parameters after 10 min of working heart perfusion, as seen in Table 7-2. Mechanical function at 10 min of resynthesis (measured as HR·PSP) was similar between control and hypertrophied hearts (28.05 ± 1.01 (n=29) *vs* 26.12 ± 0.84 (n=29) beats$\cdot$min$^{-1}\cdot$mm Hg$\cdot 10^{-3}$, respectively). However, measurements of cardiac work (measured as cardiac output·PSP) suggested that a depression in the mechanical function of the hypertrophied hearts compared to control hearts existed (55.12 ± 4.24 (n=29) *vs* 67.16 ± 4.18 (n=29) ml$\cdot$min$^{-1}\cdot$mm Hg$\cdot 10^{-2}$, respectively). This is due to the significantly decreased cardiac output of the hypertrophied hearts compared to control hearts. Mechanical function was stable throughout the 60 min period of glycogen resynthesis for both control and hypertrophied hearts. However, at the end of the 60 min period, cardiac output, aortic output, and coronary flow were all significantly depressed in the hypertrophied hearts compared to control hearts. The hypertrophied heart exhibited the same depression in mechanical function measured as cardiac work when compared to control hearts at the end of 60 min glycogen resynthesis (52.83 ± 3.63 (n=29) *vs* 67.92 ± 3.96 (n=29) $\mathrm{ml \cdot min^{-1} \cdot mm\ Hg \cdot 10^{-2}}$, respectively).

iii) Rates of glycolysis from exogenous glucose and endogenous glycogen in control and hypertrophied hearts during aerobic perfusion and low-flow ischemia

During low-flow ischemia rates of glycolysis may be in a steady state or change over the time of the ischemic insult, depending on the severity of ischemia. Plotting the recovery of glycolytic metabolism (measuring glycolytic flux from exogenous glucose during the aerobic resynthesis period, total glycolysis during low-flow, and glycolysis from exogenous glucose) *vs* time of aerobic perfusion and low-flow ischemia (Figure 7-2), demonstrates that rates of glycolysis did not vary over the 60 min period of glycogen resynthesis or low-flow ischemia (as shown by the linearity of the plotted data). Therefore, steady state rates of glycolysis from exogenous glucose during the aerobic glycogen resynthesis period and exogenous glucose and endogenous glycogen during the low-flow ischemic period can be calculated.

The hypertrophied heart exhibits steady state rates of glycolysis (Figure 7-3) that are greater than rates of glycolysis in control hearts during the aerobic glycogen resynthesis period (4446 ± 305 *vs* 2903 ± 307 nmol·min^{-1}·g dry wt^{-1}, respectively). These rates of glycolysis compare favourably to what has previously been shown in **CHAPTERS III, V, and VI**. During low-flow ischemia, the continued acceleration of glycolysis from exogenous glucose in the hypertrophied heart compared to normal hearts was not present. Rates of glycolysis from exogenous glucose during low-flow ischemia decreased significantly in the hypertrophied heart to levels that were similar in control hearts (2593 ± 503 *vs* 2797 ± 276 nmol·min^{-1}·g dry wt^{-1}, respectively). When rates of glycolysis from glycogen metabolism during low-flow ischemia were calculated (by subtracting rates of glycolysis from exogenous glucose from total rates of glycolysis), glycogen was found to make a significant contribution to total rates of glycolysis. As Figure 7-3 shows, there was a slight acceleration in the rates of glycolysis from glycogen in the hypertrophied heart compared to the control, however it was not of statistical significance (1314 ± 665 *vs* 779 ± 310 nmol·min^{-1}·g dry wt^{-1}, respectively).

iv) Rates of glucose oxidation from exogenous glucose and endogenous glycogen in control and hypertrophied hearts during aerobic and low-flow ischemia

The metabolism of glucose, via glucose oxidation is shown in Figure 7-4. The presence of measurable rates of glucose oxidation during low-flow ischemia suggests that the degree of ischemia, although severe enough to depress function, did not entirely suppress oxidative metabolism. Rates of glucose oxidation from both endogenous glycogen and exogenous glucose were severely depressed, but exhibited a linearity that was similar to what was seen for glycolysis during low-flow ischemia. This suggests that rates of glucose oxidation during low-flow ischemia were sustained over the 60 min period.

The metabolism of glucose via glucose oxidation during the aerobic resynthesis period did not show the same linearity as did glucose metabolized via glycolysis during the same period. The oxidation of glucose significantly increased in control hearts (from 518 ± 68 to 786 ± 91 nmol·min^{-1}·g dry wt^{-1}) between 10 to 20 min, and 40 to 60 min of aerobic perfusion respectively. A significant increase of greater magnitude occurred in hypertrophied hearts from (677 ± 91 to 1145 ± 124 nmol·min^{-1}·g dry wt^{-1}) during the same time periods. Because the rates of glucose oxidation do not follow a steady state rate over the 60 min period of perfusion, the initial rate (10 to 20 min of resynthesis) is taken to be indicative of the contribution of exogenous glucose to glucose oxidation. The increase in glucose oxidation at the end of the 60 min resynthesis period may be due to an increased contribution of labelled glycogen (i.e., [U-^{14}C]glucose) cycling during resynthesis, to glucose oxidation.

During the early period of aerobic resynthesis (10 to 20 min) (Figure 7-5) steady state rates of glucose oxidation are similar in the hypertrophied hearts compared to controls (677 ± 91 *vs* 518 ± 68 nmol·min^{-1}·g dry wt^{-1}). These rates of glucose oxidation are significantly higher than what has previously been measured in both hypertrophied and control hearts under similar perfusate conditions (Figure 5-6). During low-flow ischemia steady state rates of glucose oxidation decreased considerably, however there remained measurable rates of glucose oxidation from both glycogen and exogenous glucose. In control hearts, rates of glucose oxidation from exogenous glucose compared to endogenous glycogen were 175 ± 31 and 155 ± 59 nmol·min^{-1}·g dry wt^{-1}, respectively. Similar rates of glucose oxidation from exogenous glucose and endogenous glycogen are seen in the hypertrophied heart (168 ± 53 and 197 ± 94 nmol·min^{-1}·g dry wt^{-1}, respectively).

v) Metabolite content of control and hypertrophied hearts after glycogen depletion, resynthesis and low-flow ischemia

a) Glycogen content

Table 7-3 shows the glycogen content of the control and hypertrophied hearts after the 30-min period of glycogen depletion, 60 min of glycogen resynthesis and labelling, and after 60 min of low-flow ischemia. Glycogen levels in freshly frozen, unperfused hearts were 116.1 ± 11.9 (n=8) and 126.6 ± 12.2 (n=8) μmol·g dry wt^{-1}, for control and hypertrophied hearts, respectively. Glycogen was successfully resynthesized after 60 min perfusion in the presence of 1.2 mM palmitate, 0.5 mM lactate, and 11 mM glucose, as seen in Table 7-3. The percentage of the glycogen pool that was labelled, was similar in both the control and hypertrophied hearts at the end of the resynthesis period (61.6 ± 4.3% (n=7) and 54.9 ± 6.4% (n=11), respectively). At the end of the period of low-flow ischemia the glycogen pool was depleted to similar levels in both control and hypertrophied hearts.

There was also an incorporation of the label added during the low-flow period into the glycogen pool, suggesting glycogen synthesis during low-flow ischemia (16.1 ± 1.8% (n=13) and 24.1 ± 3.3% (n=9) of the glycogen pool was radiolabelled with the isotope of glucose added during low-flow ischemia in control and hypertrophied hearts, respectively). The glycogen pool at the end of low-flow ischemia was radiolabelled to a similar extent as that seen after the resynthesis period (52.3 ± 4.4% (n=13) and 62.0 ± 7.6% (n=9) of the glycogen pool in control and hypertrophied hearts, respectively). However, these numbers are indicative of radiolabel remaining after the resynthesis period as well as label that was incorporated during low-flow ischemia. Therefore a true measure of the remaining radiolabel from the resynthesis period would have to take into account the amount of label resynthesized during low-flow ischemia. This was calculated by taking the total radiolabel at the end of low-flow ischemia, and subtracting the amount of radiolabel resynthesized during low-flow ischemia. These values show that both control and hypertrophied hearts had a similar amount of the initial radiolabel (from the glycogen resynthesis period) remaining at the end of low-flow ischemia (36.2 ± 4.8% and 37.9 ± 8.3%, respectively)

b) Lactate content

Lactate content at the end of the 30-min period of glycogen depletion was similar in both control and hypertrophied hearts (Table 7-3). At the end of the 60-min resynthesis period, there was a greater tissue lactate content in the hypertrophied hearts than controls. This may have occurred due to the lower coronary flow rates in hypertrophied hearts compared to controls (51.1 ± 2 *vs* 77.8 ± 1.0 $ml \cdot min^{-1} \cdot g$ dry wt^{-1}, Table 7-2) coupled with accelerated rates of glycolysis. There was also a significant accumulation of lactate in the hypertrophied heart compared to control hearts at the end of the 60-min period of low-flow ischemia.

c) High energy phosphates

ATP values were not different in either the controls or hypertrophied hearts at the end of 30 min depletion period (11.1 ± 1.3 (n=7) *vs* 9.5 ± 0.9 (n=6) $\mu mol \cdot g$ dry wt^{-1}, respectively). Neither were levels of creatine phosphate (16.8 ± 2.3 (n=7) *vs* 14.2 ± 1.3 (n=6) $\mu mol \cdot g$ dry wt^{-1} for control and hypertrophied hearts, respectively). The 60-min period of glycogen resynthesis did not increase ATP or creatine phosphate content in control hearts (10.4 ± 1.4 (n=7) and 18.6 ± 3.7 (n=7) $\mu mol \cdot g$ dry wt^{-1}, respectively). However hypertrophied hearts had an increased creatine phosphate content (from 14.2 ± 1.3 (n=7) to 20.0 ± 1.8 (n=8) $\mu mol \cdot g$ dry wt^{-1}). ATP content did not change in the hypertrophied hearts (10.3 ± 0.9 (n=8) $\mu mol \cdot g$ dry wt^{-1}) at the end of the 60 min resynthesis period. The end of low-flow ischemia both ATP and creatine phosphate decreased in control hearts (8.0 ± 1.4 (n=6) and 13.4 ± 2.6 (n=6) $\mu mol \cdot g$ dry wt^{-1}, respectively), and in hypertrophied hearts (7.4 ± 1.2 (n=6) and 10.0 ± 2.7 (n=6) $\mu mol \cdot g$ dry wt^{-1}, respectively).

vi) Calculated rates of glycogenolysis

When rates of glycogen depletion were calculated over the 60-min period of low-flow ischemia by subtracting the glycogen content at the end of ischemia from the glycogen content after resynthesis (from Table 7-3), these calculated rates of glycogen breakdown compare favourably to those measured using the technique of glycogen labelling developed in this chapter. In controls the calculated rates of glycogen use were 1160 ± 190 *vs* 780 ± 310 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{g dry wt}^{-1}$ for the direct measurement. In hypertrophied hearts the values were also similar; the calculated rate of glycogen use being 1030 ± 150 *vs* 1310 ± 670 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{g dry wt}^{-1}$ using direct measurements employed by this study.

D) Summary

From the results of this study it can be concluded that;

1) catabolism of glucose from glycogen is a significant component of the total rates of glycolysis during a low-flow ischemia, in both normal and hypertrophied rat heart,

2) the contribution of exogenous glucose to glycolysis during a low-flow ischemia, is not significantly different in the hypertrophied heart compared to normal rat heart,

3) rates of glycolysis from exogenous glucose decrease significantly in the hypertrophied heart compared to rates of glycolysis from exogenous glucose during aerobic perfusion,

4) residual rates of glucose oxidation during a low-flow ischemia are similar regardless of whether the source is exogenous glucose and endogenous glycogen,

5) the contribution of exogenous glucose to total rates of glycolysis is nearly 3-fold greater in both the control and hypertrophied heart compared to the contribution of glycogen to glycolysis,

6) glycogen preferentially supports glucose oxidation, even during a situation of low-flow ischemia where oxidative metabolism is severely depressed.

Table 7-1: Heart and body weights of control and aortic banded animals

	Dry Heart Weight (g)	Wet Heart Weight (g)	Body Weight (g)	Heart Weight/Body Weight Ratio (x 10^{-3})
Control (n=36)	0.36 ± 0.02	1.78 ± 0.03	433 ± 6	4.1 ± 0.1
Hypertrophied (n=38)	$0.47 \pm 0.02^*$	$2.16 \pm 0.04^*$	433 ± 6	$5.0 \pm 0.1^*$

[*] significantly different from control value ($p \leq 0.05$).

Table 7-2: Functional parameters of control and hypertrophied hearts at 10 min and 60 min of the glycogen resynthesis period

Parameter Measured	Control (n=29) 10 min	60 min	Hypertrophied (n=29) 10 min	60 min
Heart Rate (beats·min^{-1})	234 ± 8	270 ± 5[†]	220 ± 8	257 ± 6[†]
Peak Systolic Pressure (mm Hg)	120 ± 2	115 ± 2	119 ± 2	114 ± 2
Developed Pressure (mm Hg)	57 ± 3	49 ± 3	52 ± 3	43 ± 2[†]
Cardiac Output (ml·min^{-1})	55 ± 3	58 ± 3	46 ± 3[*]	46 ± 3[*]
Aortic Output (ml·min^{-1})	29 ± 2	30 ± 2	24 ± 2	24 ± 2[*]
Coronary Flow (ml·min^{-1})	26 ± 1	28 ± 1	24 ± 2	24 ± 2[*]
Coronary Flow (ml·min^{-1}·g dry wt^{-1})	77 ± 1	78 ± 1	51 ± 2[*]	51 ± 2[*]

[*] significantly different from control value ($p \leq 0.05$).
[†] significantly different from comparable 10 min value ($p \leq 0.05$).

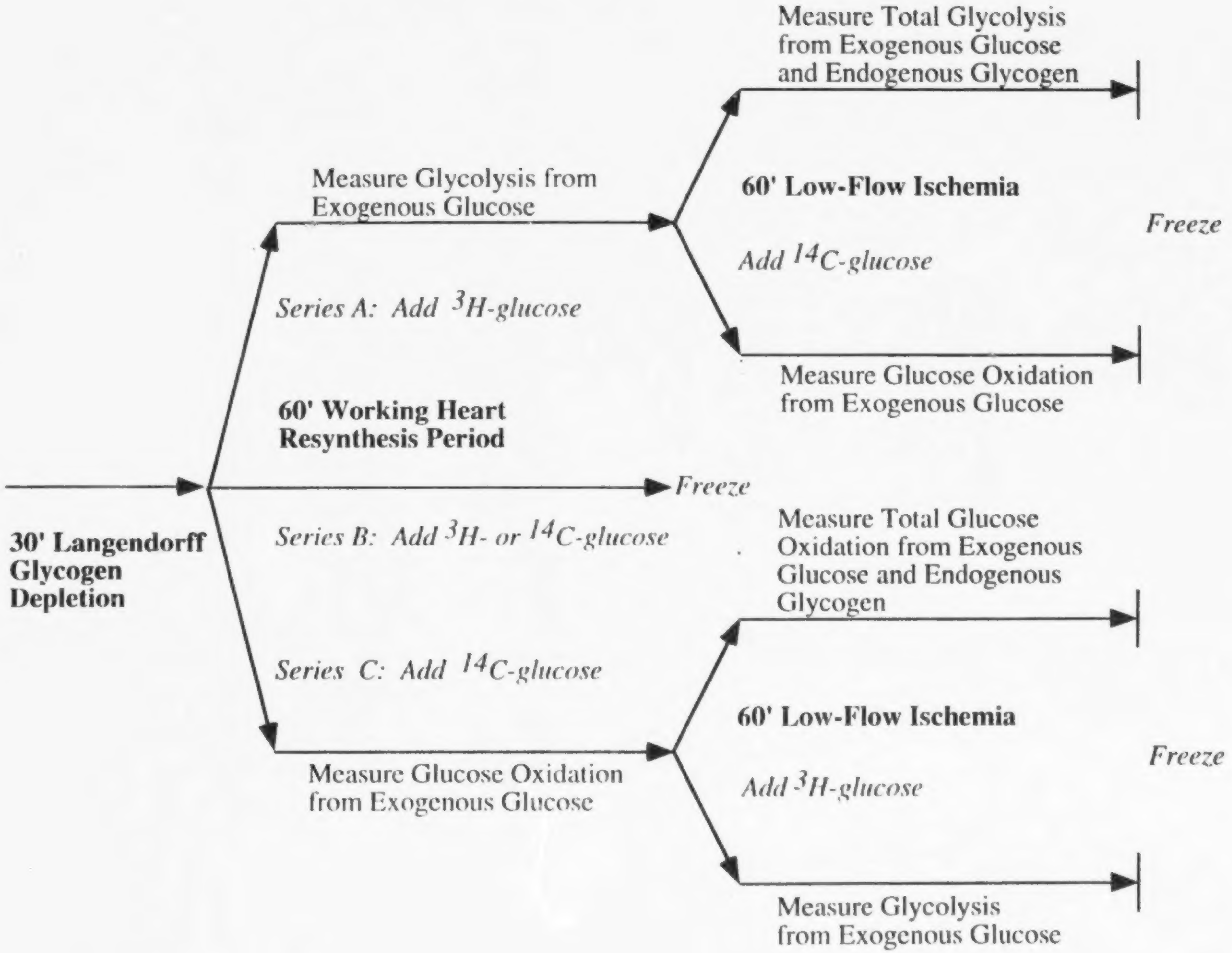
Table 7-3: Myocardial glycogen and lactate of control and hypertrophied hearts at the end of the 30 min depletion period, 60 min resynthesis period, and 60 min lo-flow period

	Glycogen	Lactate
μmol·g dry wt^{-1}		
30 min Depletion		
Control	$51.0 \pm 4.5^{\dagger}$ (7)	$1.5 \pm 0.4^{\dagger}$ (5)
Hypertrophied	$50.3 \pm 3.4^{\dagger}$ (9)	$1.0 \pm 0.2^{\dagger}$ (7)
60 min Resynthesis		
Control	120.7 ± 10.4 (7)	4.3 ± 0.6 (7)
Hypertrophied	112.3 ± 8.0 (11)	$10.8 \pm 1.8^{*}$ (7)
60 min Low-flow Ischemia		
Control	$48.6 \pm 5.1^{\dagger}$ (19)	$26.9 \pm 3.3^{\dagger}$ (7)
Hypertrophied	$46.3 \pm 5.6^{\dagger}$ (17)	$42.3 \pm 5.7^{*\dagger}$ (7)

[*] significantly different from control value ($p \leq 0.05$).

[†] significantly different from comparable 60 min resynthesis period value ($p \leq 0.05$).

Figure 7-1: Perfusion protocol used to deplete, resynthesize, radiolabel, and measure metabolism of glycogen in control and hypertrophied rat hearts. After 30 min of no substrate langendorff perfusion, the hearts were put into working mode with perfusion buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100 $\mu U \cdot ml^{-1}$ insulin. Series A hearts were additionally pulsed with [5-^{3}H]glucose. This allowed for the labelling of the glycogen pool as it was resynthesized during the 60 min working heart mode. After 60 min, [U-^{14}C]glucose was added to the perfusion medium and a low-flow ($0.5\ ml \cdot min^{-1}$) ischemia was produced for 60 min after which time the hearts were frozen. Series B hearts had either [5-^{3}H] or [U-^{14}C]glucose added to the perfusion medium and were frozen at the end of the 60 min glycogen resynthesis period. Series C hearts were similar to series A in that a low-flow ischemia was produced at the end of the 60 min glycogen resynthesis period, but the isotope used was added in the opposite order of Series A hearts; ie [U-^{14}C]glucose added first, followed by [5-^{3}H]glucose just prior to low-flow ischemia.


30' Langendorff Glycogen Depletion
60' Working Heart Resynthesis Period
Series A: Add ^{3}H-glucose
Measure Glycolysis from Exogenous Glucose
Series B: Add ^{3}H- or ^{14}C-glucose
Freeze
Series C: Add ^{14}C-glucose
Measure Glucose Oxidation from Exogenous Glucose
60' Low-Flow Ischemia
Add ^{14}C-glucose
Measure Total Glycolysis from Exogenous Glucose and Endogenous Glycogen
Measure Glucose Oxidation from Exogenous Glucose
Freeze
60' Low-Flow Ischemia
Add ^{3}H-glucose
Measure Total Glucose Oxidation from Exogenous Glucose and Endogenous Glycogen
Measure Glycolysis from Exogenous Glucose
Freeze

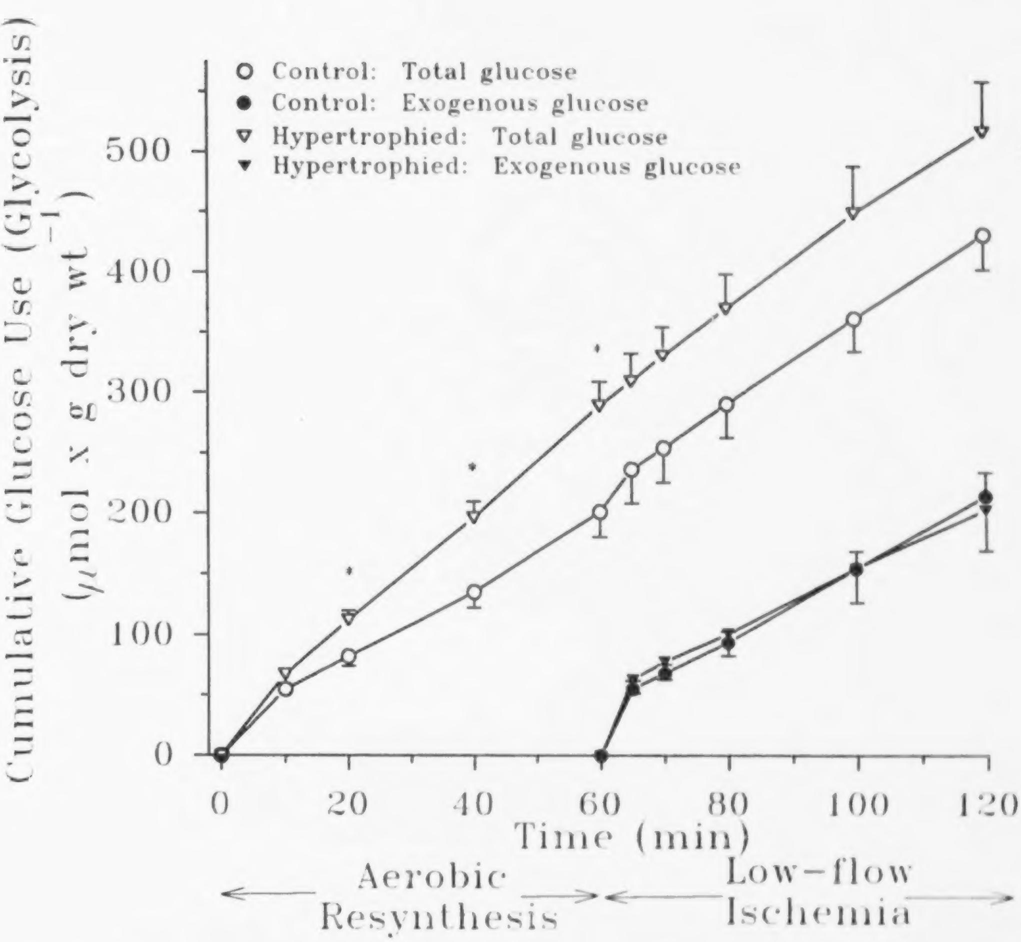


Figure 7-2: Cumulative glucose use (glycolysis) in control and hypertrophied rat hearts during the 60 min glycogen resynthesis period and during low-flow ischemia. Hearts perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and $100\mu U \cdot ml^{-1}$ insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. Total cumulative glucose use (glycolysis) during the 60 min glycogen resynthesis period and during ischemia was measured in hearts designated as Series A (see Figure 7-1). Cumulative glucose use (glycolysis) from exogenous glucose during ischemia was measured in hearts designated as Series C. ^3H_2O (from [5-^{3}H]glucose) production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=17 (controls Series A), n=12 (hypertrophied Series A), n=10 (controls Series C), and n=8 (hypertrophied Series C).

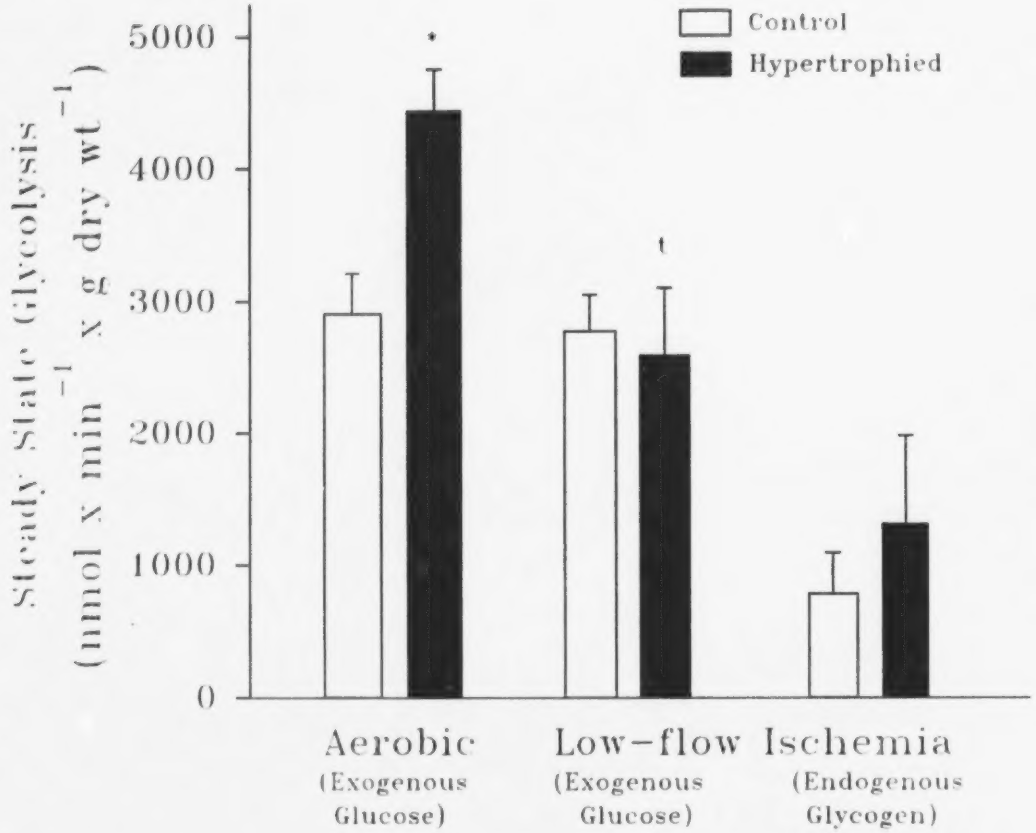


Figure 7-3: Steady state rates of glycolysis in control and hypertrophied rat hearts during the 60 min glycogen resynthesis period and during low-flow ischemia. Hearts were perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and 100μU·ml^{-1} insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. Steady state rates of glycolysis during the glycogen synthesis period was measured from hearts designated Series A (see Figure 7-1). Series C hearts measured steady state rates of glycolysis from exogenous glucose during ischemia. Subtraction of steady state glycolysis rates during ischemia from total steady state glycolysis rates (Series A) during ischemia allowed for the calculation of endogenous glycogen to steady state glycolysis during ischemia. ^3H_2O production (from [5-^{3}H]glucose) was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=17 (control Series A), n=12 (hypertrophied Series A), n=9 (control Series C), and n=9 (hypertrophied Series C). *significantly different from control hearts ($p \leq 0.05$). ^{t}significantly different from comparable aerobic value ($p \leq 0.05$).

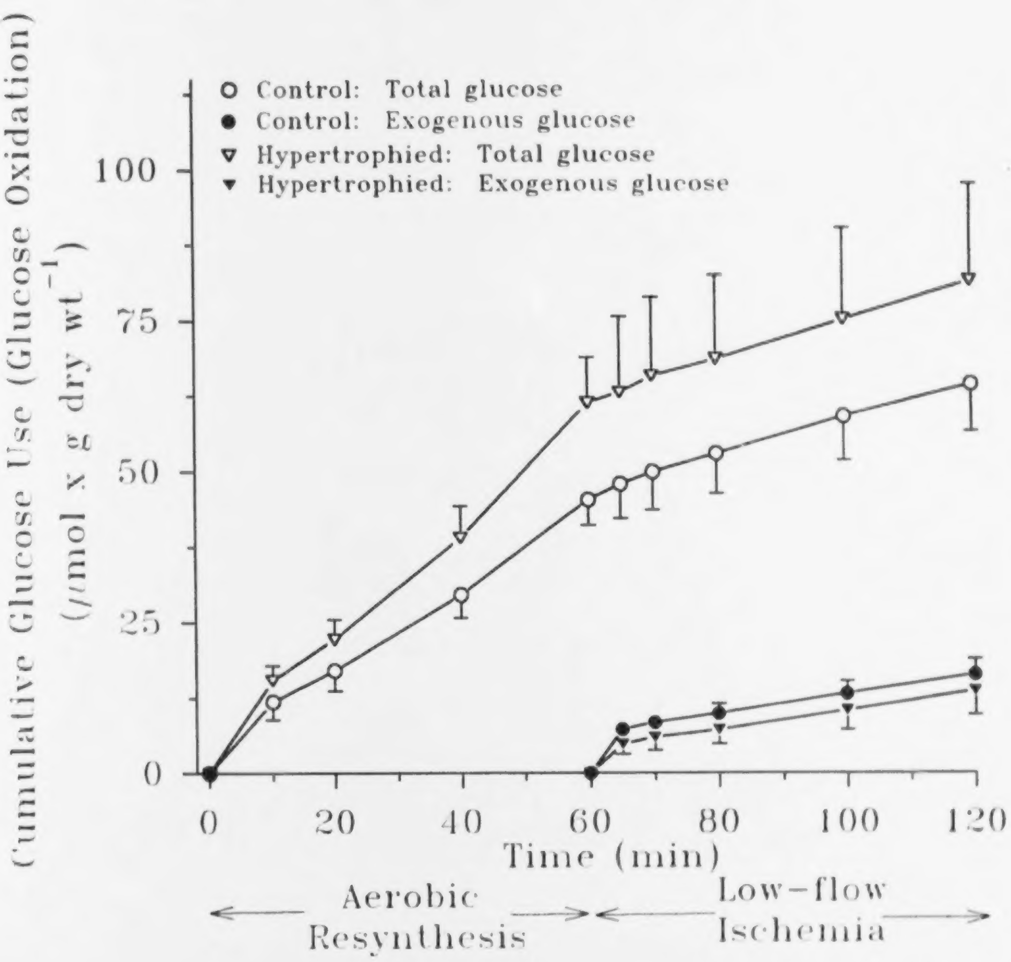


Figure 7-4: Cumulative glucose use (glucose oxidation) in control and hypertrophied rat hearts during the 60 min glycogen resynthesis period and during low-flow ischemia. Hearts perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and $100\mu U \cdot ml^{-1}$ insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. Total cumulative glucose use (glucose oxidation) during the glycogen resynthesis period and during ischemia was measured in hearts designated as Series C (see Figure 7-1). Cumulative glucose use (glucose oxidation) from exogenous glucose during ischemia was measured in hearts designated as Series A. $^{14}CO_2$ (from [U-^{14}C]glucose) production was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=12 (control Series C) n=17 (hypertrophied Series C), n=9 (controls Series A), and n=9 (hypertrophied Series A).

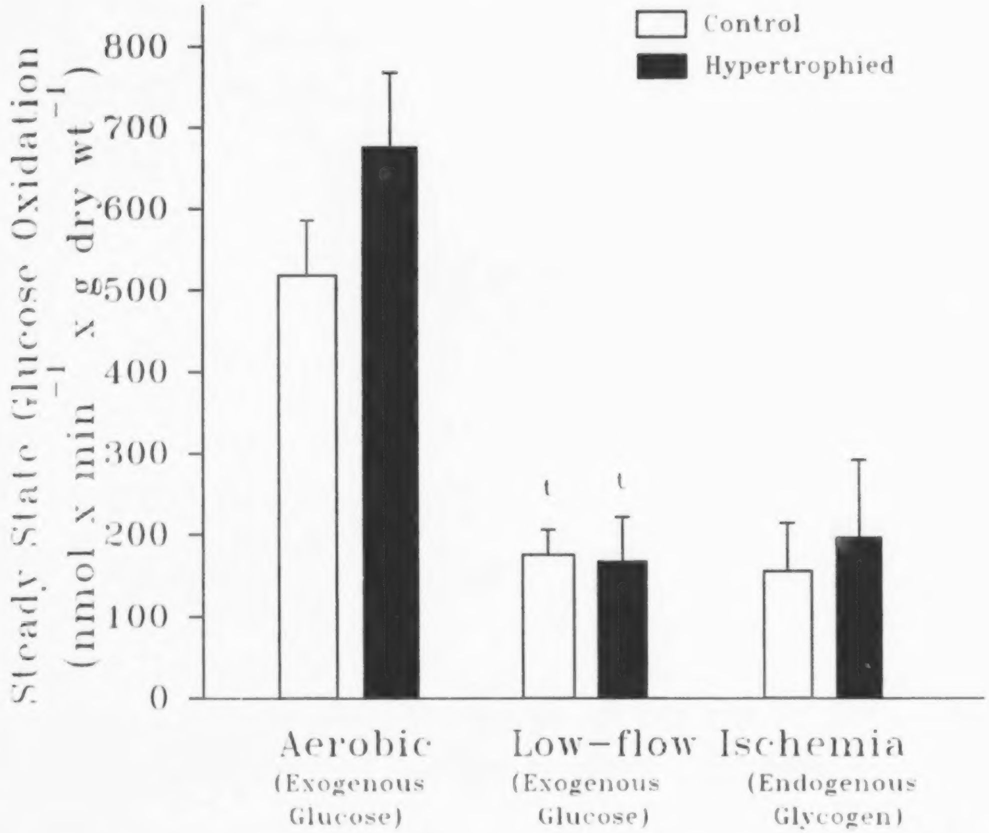


Figure 7-5: Steady state rates of glucose oxidation in control and hypertrophied rat hearts during the 60 min glycogen resynthesis period and during low-flow ischemia. Hearts were perfused with buffer containing 11 mM glucose, 0.5 mM lactate, 1.2 mM palmitate, and $100\mu U \cdot ml^{-1}$ insulin at a left atrial filling pressure of 11.5 mm Hg and an afterload pressure of 80 mm Hg. Steady state rates of glucose oxidation during glycogen resynthesis period was measured from hearts designated Series C (see Figure 7-1). Series A hearts allowed the measurement of steady state rates of glucose oxidation from exogenous glucose during ischemia. Subtraction of steady state glucose oxidation rate during ischemia from total steady state glucose oxidation rates (Series C) during ischemia allowed for the calculation of endogenous glycogen to steady state glucose oxidation during ischemia. $^{14}CO_2$ production (from [U-^{14}C]glucose) was measured as described in the **CHAPTER II**. Values are the mean ± SEM, n=12 (control Series C), n=17 (hypertrophied Series C), n=9 (control low-flow Series A), and n=8 (hypertrophied Series A). ^{t}significantly different from comparable aerobic value ($p \leq 0.05$).

CHAPTER VIII

DISCUSSION

Despite the fact that alterations in myocardial metabolism have been implicated as a contributing factor to the pathophysiology of hypertrophic heart disease, a direct and complete determination of myocardial energy substrate preference has not previously been made. Measurements of glycolysis, glucose, lactate and fatty acid oxidation in isolated working hearts perfused with physiological and pathophysiological levels of fatty acids, glucose, and lactate, allow for the direct determination of energy substrate preference of the hypertrophied heart. Since myocardial work is a major determinant of energy substrate use (Neely et al., 1976), it was necessary to consider first the effects of varying work states on energy substrate use in the hypertrophied heart.

A) Energy substrate use in the hypertrophied rat heart under conditions of changing work load

Under aerobic conditions glycolysis was not impaired in the hypertrophic heart and was, in fact, accelerated under conditions of low work. Furthermore, the decreased fatty acid oxidation rates that were seen, and which have previously reported to occur in hypertrophied hearts by El Alaoui-Talibi et al. (1992), occurred primarily at low workloads. This decrease in fatty acid oxidation became less evident as workload increased. The decrease in ATP production from fatty acid oxidation in hypertrophied hearts was partially compensated for by an increased contribution of glycolytic rates to ATP production. It can also be seen that carbohydrate oxidation was not impaired in the hypertrophied heart, and finally that following an increase in myocardial work, a marked increase in carbohydrate oxidation was the main extra source of ATP production in both control and hypertrophied hearts.

The controversy that exists concerning the glycolytic ability of hypertrophic hearts was directly addressed by the data in this thesis. Numerous previous studies have indirectly suggested that the glycolytic capacity is greater in the hypertrophied heart than in the normal heart (Bishop and Aultshuld, 1971; Ingwall, 1984; Taegtmeyer and Overturf, 1988; Anderson et al., 1990ab; Bladergroen et al., 1990; Allard 1994b). It has also been shown that the myocardium from hypertrophied hearts produces more lactate during hypoxia (Bishop and Aultshuld, 1971; Anderson et al., 1990ab) and ischemia (Bladergroen et al., 1990) than do normal hearts. On the other hand, Cunningham et al (1990) have suggested that hypertrophied hearts have a reduced capacity to recruit anaerobic glycolysis to preserve mechanical function during hypoxia. Previous studies by this group however, have focused on measuring either myocardial lactate levels at the end of an ischemic period, or lactate production by the heart. Since lactate itself is an important oxidative substrate of the heart, it was important to directly measure glycolysis under conditions in which lactate oxidation was also measured. The findings in this thesis demonstrate that glycolysis is *not* impaired in the hypertrophied heart and under most experimental conditions is accelerated. Whether the enhanced glycolytic rates seen in hypertrophied hearts are due to an actual increase in glycolytic capacity or whether they occur secondary to decreased ATP production from fatty acid oxidation cannot however be definitively concluded.

Of interest was the finding that the myocardium of both control and hypertrophied hearts oxidizes substantial quantities of lactate (Figure 3-2). This finding is consistent with those of Gertz et al., (1981) who demonstrated that at least 85% of the lactate extracted by the myocardium is oxidized. Their study also showed that the myocardium can release lactate at the same time lactate is being extracted and oxidized by the myocardium. The data presented here are entirely consistent with this finding. Rates of glycolysis in both control and hypertrophied hearts were found to be much greater than the rates of glucose oxidation (Figure 3-1) and, as such, lactate production occurs since not all the glucose passing through glycolysis was oxidized.

An additional finding of this study was that the steady state rates of lactate oxidation were similar in both the control and hypertrophied hearts (Figure 3-2) as were the rates of glucose oxidation (Figure 3-1). This suggests that uptake of pyruvate into the mitochondria and passage through the PDH complex to form acetyl-CoA is not impaired in the hypertrophied heart. In fact, as was seen in the control hearts, following an increase in workload, the extra acetyl-CoA necessary for the TCA cycle was derived almost exclusively from the PDH complex. In neither experimental group (control or hypertrophied hearts) was any increase in β-oxidation of fatty acids seen following the transition to high work.

Steady state rates of myocardial palmitate oxidation were significantly decreased in the hypertrophied heart compared to the control heart during low work conditions

(Figure 3-3). This finding is consistent with reports by other investigators who have shown reduced levels of long-chain fatty acid oxidation in mitochondria isolated from hypertrophied-failing guinea pig hearts (Wittels and Spann, 1968) or in intact volume-overloaded hypertrophied rat hearts (El Alaoui-Talibi et al., 1992; Cheikh et al., 1994). The reduction in palmitate oxidation in the hypertrophied hearts was associated with lower levels of myocardial L-carnitine (Table 3-4). A number of investigators have also reported that myocardial L-carnitine content is reduced in hypertrophied hearts (Reibel et al., 1983; El Alaoui-Talibi et al., 1992) and have shown that decreased myocardial L-carnitine is associated with impaired fatty acid oxidation and impaired left ventricular function (El Alaoui-Talibi et al., 1992). L-carnitine plays an essential role in the oxidation of long-chain fatty acids. The enzymes CPT I and II, present on the outer and inner mitochondrial membrane, respectively, require L-carnitine for the reversible formation of L-carnitine esters of long-chain fatty acids in order to transfer the acyl group to coenzyme-A (CoA) within the mitochondrial matrix (Fritz et al., 1962). The inner mitochondrial membrane is impermeable to long-chain acyl-CoA (Pandle and Parvin, 1976) and, as such, L-carnitine is absolutely essential for the translocation of the acyl group across the mitochondrial inner membrane to the site of β-oxidation. The reduced oxidation of long-chain fatty acids in the hypertrophied heart may be a consequence of reduced myocardial L-carnitine levels.

Determination of steady state myocardial ATP production from the various exogenous substrates during the period of low-work confirmed previous reports (Neely and Morgan, 1974; Saddik and Lopaschuk, 1991) that palmitate is the preferred energy substrate of the normal heart (Figure 3-2). Shown for the first time is evidence that palmitate is also the preferred exogenous energy substrate of the hypertrophied heart under conditions of low-work (Figure 3-2). However, the relative proportion of total ATP production derived from palmitate oxidation was less in the hypertrophied heart than the control heart (Figure 3-5). At the same time, the proportion of ATP produced by glycolysis was substantially greater in the hypertrophied heart than in control hearts (Figure 3-5). This finding suggests that the hypertrophied myocardium responds to the impaired oxidation of fatty acids by a compensatory increase in glycolysis. This pattern of exogenous substrate use is similar to that of the early neonatal heart where systems to oxidize fatty acids have not yet matured and the heart is extremely dependent upon glycolysis as a source of ATP production (Lopaschuk et al., 1991). The shifts in isoenzymes of lactate dehydrogenase (Bishop and Aultschuld, 1971) and creatine kinase (Ingwall, 1984) towards more fetal, anaerobic forms and the increased glycolytic enzyme activity (Taegtmeyer and Overturf, 1988) in the hypertrophied heart are consistent with this viewpoint.

Total ATP production from exogenous energy substrates was less in the hypertrophied hearts than the control hearts (Figure 3-4). This decreased total ATP production was present despite an absence of significant differences in myocardial energy status between the control and the hypertrophied hearts. This finding suggests that some other unmeasured energy substrate was being used by the hypertrophied hearts. Previous studies have shown that a substantial portion of ATP production in the normal heart is derived from oxidation of endogenous lipids (Saddik and Lopaschuk, 1991). A greater proportion of ATP production from endogenous lipids by the hypertrophied heart than the normal heart could account for the apparent decreased steady state rate of ATP production from exogenous substrates in the hypertrophied heart. The contribution of endogenous lipids (triacylglycerols) to overall ATP production was not measured in this study, therefore their exact contribution to overall ATP production is unknown.

During periods of high work, both the control and hypertrophied hearts showed substantially increased rates in the oxidation of glucose and lactate (Figure 3-1 and 3-2). The proportion of ATP derived from glucose oxidation increased more than two-fold in the hypertrophied hearts and slightly less than two-fold in the control hearts. The ATP produced from lactate oxidation increased nearly 89% in the control hearts and approximately 53% in the hypertrophied hearts. This increased oxidation of carbohydrates was associated with no change in the rate of palmitate oxidation in both groups such that glucose became the preferred exogenous energy substrate of the hypertrophied heart. Other reports have shown that perfusion of normal isolated hearts with buffer containing fatty acids results in an inhibition of glucose oxidation (Newsholme et al., 1962; Neely and Morgan, 1974; Ingwall, 1984; Kereby et al., 1985; Saddik and Lopaschuk, 1991). This inhibition of glucose oxidation is believed to be a consequence of increased acetyl-CoA/CoA and NADH/NAD$^+$ ratios within the mitochondrial matrix that decrease PDH complex activity (Kerbey et al., 1985; Patel and Roche, 1990). With an increased workload and a greater demand for acetyl-CoA, the intramitochondrial ratio of acetyl-CoA/CoA and NADH/NAD$^+$ likely decreased which removed the inhibition of the PDH complex and resulted in an acceleration of the rate of glucose oxidation. Increases in cytosolic Ca^{2+} may also have resulted in an increased activation of PDH, and therefore increased rates of carbohydrate oxidation. The finding of a marked stimulation of oxidation of glucose and lactate in response to an increased workload is in contrast to a study by Neely et al., (1976) which showed that although glucose oxidation had a direct linear relationship with cardiac work, so did the oxidation of palmitate. A major difference between the two studies is that the levels of cardiac work in the study by Neely et al., (1976) were substantially higher than in the present study. A preferential increase in glucose use compared to palmitate oxidation has also been recently demonstrated in isolated working normal hearts exposed to epinephrine (Collins-Nakai et al., 1994) and to increased levels of calcium (Schönekess et al., 1995c), as well as in humans with exercise (Gertz et al., 1988). The level of cardiac work, expressed as the HR·PSP product, in those two studies was comparable to those achieved in the **CHAPTER III** of this thesis. Thus, an immediate need for increased energy requirements associated with mild increases in cardiac work appear to be met preferentially by the utilization of carbohydrates rather than fatty acids in both normal and hypertrophied hearts.

Myocardial hypertrophy resultant of a pressure-overload yields lower rates of fatty acid oxidation and higher rates of glycolysis than normal hearts. Despite decreased rates of palmitate oxidation, it remains the preferred exogenous energy substrate of the hypertrophied heart during low-work conditions. An increased energy demand associated with modest elevations in cardiac work is met by substantial increases in the rates of carbohydrate (glucose and lactate) oxidation in both control and hypertrophied hearts such that carbohydrates become the preferred energy substrate of the hypertrophied heart under these conditions. A reduction of L-carnitine in hypertrophied myocardium does not interfere with carbohydrate oxidation, and glycolysis is not decreased in the hypertrophied heart and is actually increased at low-workloads.

B) The contribution of fatty acids and lactate to overall ATP production in normal rat hearts

It was clear by the initial findings described in this thesis, that even though glycolysis uncoupled from glucose oxidation yields lactate that is released, extracellular lactate can be simultaneously taken up and oxidized. There are a number of studies that have shown that there is a net chemical extraction of lactate in human hearts (Gertz et al., 1981, 1988), and lactate release during ischemia (Guth et al., 1990), increased work states (Massie et al., 1994), and hypoxia (Mazer et al., 1989). In fact, the presence of lactate and fatty acids does have an effect on the rate of utilization of either lactate or fatty acids (Liu and Spitzer, 1978), as well as the utilization of glucose (Saddik and Lopaschuk, 1991). Drake et al. (1980) suggested that when the normal dog heart was exposed to high levels of fatty acids or glucose, the preferred source of energy substrate was lactate. What has been shown in this thesis is that lactate oxidation is effectively inhibited by increased amounts of fatty acids, but also accelerates as the concentration of lactate increases (Figure 4-5). Varying lactate concentrations has a minimal effect on glycolysis at 0.4 mM palmitate. However at 1.2 mM palmitate, a concentration that will effectively inhibit glycolysis (Saddik and Lopaschuk, 1991), increased concentrations of lactate actually relieve this inhibition (Figure 4-3). Only at 0.4 mM palmitate was there no effect of increasing lactate concentrations on the rates of glycolysis. This may have been due to glycolytic rates already being "maximal" in a low-fat (0.4 mM palmitate) situation (Saddik and Lopaschuk, 1991; Lopaschuk and Saddik, 1993).

Glucose oxidation was not inhibited to any great extent by either fatty acids or lactate in this study, except at 8.0 mM lactate where there was a significant difference between rates of glucose oxidation in the 0.4 mM and 1.2 mM palmitate group (Figure 4-4). Lactate oxidation dramatically increased as the availability increases. Increases of 4- to 5-fold in the 0.4 mM palmitate group, and almost 7- to 8-fold in the 1.2 mM palmitate group occurred. However, in absolute terms, rates of lactate oxidation were considerably depressed in the 1.2 mM palmitate group compared to the 0.4 mM palmitate group. Palmitate oxidation also increased as the external concentration of lactate increased (Figure 4-6). There was significantly less exogenous fatty acids oxidized at 0.4 mM palmitate compared to 1.2 mM palmitate, a finding which has been shown previously (Saddik and Lopaschuk, 1991).

When rates of ATP production were calculated from steady state rates of substrate oxidation and glycolysis, lactate was found to contribute significantly in the 0.4 mM palmitate group (Figure 4-7). The contribution of lactate to total ATP production increases from 17% at 0.5 mM lactate to 37% at 8.0 mM, with the majority of ATP production coming from fatty acid oxidation (81% at 0.5 mM and 52% at 8.0 mM lactate). At 1.2 mM palmitate, the contribution of lactate to ATP production was dramatically reduced to 3% at 0.5 mM and 13% at 8.0 mM lactate. Therefore it is clear that only at low levels of fatty acids (0.4 mM palmitate) does lactate contribute a relatively large proportion of the hearts ATP supply (Figure 4-8). High levels of exogenous fatty acids will effectively contribute over 80% of the hearts ATP supply, even at high levels of exogenous lactate.

It is difficult to reconcile why an increase in the total rates of ATP production was seen. It may be due to a substrate-induced increase in ATP production (Katz et al., 1989). When O_2 consumption was measured, the actual rates of O_2 consumed were higher than the rates calculated from the steady state rates of exogenous substrate oxidized in the 0.5 mM lactate and 0.4 mM palmitate group, but agreed reasonably well in the other groups. The fact that endogenous sources of energy for ATP production are contributing to overall O_2 consumption, may be a possible explanation for what occurs in the 0.5 mM lactate and 0.4 mM palmitate group. It has previously been shown that endogenous triacylglycerols can contribute significantly to the production ATP, especially when exogenous levels of fatty acids are low (Saddik and Lopaschuk, 1991; Lopaschuk and Saddik, 1993). Data presented in this thesis confirms this and shows that hearts perfused for 100 min at 0.5 mM lactate and 0.4 mM palmitate have a myocardial triacylglycerol pool that is decreased compared to hearts that were supplied with 1.2 mM palmitate (Table 4-3). Myocardial glycogen is a source of glucose that can contribute to aerobic ATP production (Goodwin and Taegtmeyer, 1995; Henning et al., 1995). However, the glycogen pool was not significantly changed between groups. Infact, it is significantly increased in size from unperfused hearts. This suggests that the differences in rates of O_2 consumption which were measured compared to what was calculated from the steady state rates of substrate oxidation in the 0.5 mM lactate and 0.4 mM palmitate group, may be due to triacylglycerol consumption (Saddik and Lopaschuk, 1991).

A question of myocardial efficiency in these hearts comes to mind when the amount of ATP produced per unit mechanical function performed is compared. The appearance of hearts perfused with 0.4 mM palmitate being more efficient than hearts perfused with 1.2 mM palmitate, may be due to the contribution of endogenous triacylglycerol to ATP production. The triacylglycerol content was decreased in the 0.5 mM lactate and 0.4 mM palmitate group and was not taken into account when ATP production was calculated. This however assumes that the decreased content of triacylglycerol is due to breakdown, and not an increased synthesis in the other groups. A substrate-induced acceleration of ATP production may be an explanation for this increase in ATP production when there was an increased availability of exogenous substrates (Katz et al., 1989). There was found to be no change in the amount of oxygen consumed when normalized per unit mechanical function in any of the groups used for this study. However, there was an increase in the amount of ATP being produced, suggesting that there was an increased efficiency in the amount of ATP being produced per unit oxygen consumed at the end of the perfusion series.

The depression in function that occurs at the end of the 100 min perfusion period may be due to the presence of lactate interfering with intracellular pH regulation, and a competition of H^+ with myofibril binding of Ca^{2+}. In all of these perfusions a Ca^{2+} concentration of 1.25 mM was used. This may not have been an adequate concentration to maintain effective mechanical function, in the light of a high or increasing extracellular lactate. It has been shown that a lactate/H^+ co-transport system exists in the heart (Mann et al, 1985; Trosper and Philipson, 1987, 1989; Poole et al., 1990; Wang et al., 1993). If a high extracellular lactate concentration was present, it may inhibit lactate/H^+ transport and thus cause an acidification of the mycocytes. An increased acidification may effectively compete with intracellular Ca^{2+} at the level of myofibril contraction, and decrease the mechanical work of the heart (Thompson et al., 1990). Myocardial efficiency may also be altered in these hearts, as an increased acidification may lead to increased Na^+/H^+ and Na^+/Ca^{2+} exchange (Tani and Neely, 1990), and result in an increased use of ATP for the maintenance of ion gradients. However, it is interesting to note that over the 100 min perfusions with 1.2 mM palmitate and either 0.5 or 8.0 mM lactate, mechanical function was maintained to a greater extent compared to the hearts perfused with 0.4 mM palmitate. The lower availability of exogenous fatty acids, may have caused an energy deficit over the 100 min period. However, this is unlikely since rates of substrate oxidation are high in all perfusion conditions. Therefore an altered mechanical efficiency or altered efficiency in the use of ATP produced by the hearts may be a cause of the depression in function seen at the end of the perfusions.

It can be concluded from this study that ATP production in the isolated working rat heart supplied with lactate and fatty acids results primarily from the oxidation of exogenous fatty acids. Lactate will contribute significantly to ATP production, only when the exogenous concentration of lactate is high, and the exogenous concentration of fatty acids (palmitate) is low. Minimal effect of varying concentrations of lactate exists on rates of glycolysis and glucose oxidation, and there seems to be a greater effect by the presence of fatty acids on glucose metabolism than by lactate.

C) Myocardial hypertrophy and energy substrate use during post-ischemic reperfusion

The significance of the metabolic alterations that exist in the hypertrophied heart, may have a part in the pathophysiology of hypertrophic heart disease. Previous studies have suggested that lactate production due to anaerobic glycolysis in hypertrophied hearts increases both during hypoxia (Bishop and Aultschuld, 1971; Anderson et al., 1990a) and during ischemia (Anderson et al., 1990a). However, it has also been suggested that the hypertrophied heart may have decreased glycolytic rates during and following hypoxia (Cunningham et al., 1990). Yet other reports suggests that the hypertrophic heart in failure has a reduced capacity to recruit anaerobic glycolysis, and this may contribute to diastolic dysfunction during ischemia and reperfusion (Gaasch et al., 1990). By directly measuring glycolytic rates (Figure 3-1) data has been presented which shows that hypertrophied hearts have increased rates of glycolysis when perfused with concentrations of free fatty acids normally seen under physiological conditions (i.e., at 0.4 mM palmitate). As shown in Figure 5-4 a significant increase in glycolytic rates is also seen in hypertrophied hearts perfused with the high concentrations of free fatty acids (1.2 mM palmitate) that can be seen during and following clinical situations of myocardial ischemia (Oliver et al., 1968; Allison et al., 1969; Opie 1975; Lopaschuk et al., 1994). The increase in glycolysis may be linked to the upregulation of some key glycolytic enzymes (Ingwall, 1984; Taegtmeyer and Overturf, 1988) or it may be an attempt to normalize ATP production (**CHAPTER III**). Despite the controversy as to whether or not the hypertrophied heart is able to recruit anaerobic glycolysis during hypoxia and post-ischemic reperfusion (Cunningham et al., 1990; Gaasch et al., 1990). data in this thesis directly demonstrates that rates of glycolysis are significantly elevated during post-ischemic reperfusion of hypertrophied rat hearts (Figure 5-4), and that they recover to a similar extent as control hearts (Figure 5-2).

Data are also presented that clearly show oxidative metabolism of both carbohydrates and fatty acids recovers to levels that are comparable, if not enhanced to pre-ischemic rates (Figures 5-2 to 5-8). Even though oxidative metabolism and glycolysis recover to above pre-ischemic rates and therefore ATP production has recovered in the hypertrophied heart (Figure 5-11), post-ischemic mechanical function remains impaired (Figure 5-1). I propose that the key to depressed functional recovery during post-ischemic reperfusion lies in the alterations that exist in glucose metabolism, namely increased rates of glycolysis uncoupled from an already depressed rate of glucose oxidation.

In non-hypertrophied hearts, oxidative metabolism recovers to pre-ischemic levels during post-ischemic reperfusion (Lopaschuk et al., 1990). However, this does not always lead to a full recovery of mechanical function. Improvements in mechanical function of hearts during post-ischemic reperfusion can be accomplished by improving the coupling between glucose oxidation and glycolysis (McVeigh and Lopaschuk, 1990;

Lopaschuk et al., 1993). While a rapid recovery of glycolysis is necessary for optimal recovery of heart function (Apstein et al., 1976, 1978, 1983; Eberli et al., 1991; du Toit and Opie, 1992; Jeremy et al., 1992; Vanoverschelde et al., 1994) high glycolytic rates uncoupled from glucose oxidation have also been linked to depressed post-ischemic functional recovery of normal hearts (Mickle et al., 1986; Liedtke et al., 1988a; Lopaschuk et al., 1993). This uncoupling of glucose oxidation from glycolysis may be due to the inhibition of the PDC by a high intramitochondrial acetyl-CoA/CoA ratio (Patel and Roche; 1990). The intra-mitochondrial acetyl-CoA/CoA ratio is influenced by the β-oxidation of fatty acids which supply the majority of overall ATP in the heart (Neely and Morgan, 1974; Saddik and Lopaschuk 1991). The presence of high levels of circulating fatty acids similar to those seen during myocardial infarction and myocardial bypass surgery, result in a greater inhibition of PDC due to an elevated intra-mitochondrial acetyl-CoA/CoA ratio (Neely and Morgan, 1974). This in turn substantially inhibits rates of glucose oxidation (Lopaschuk et al., 1990, 1993). Therefore, due to the inhibition of PDC by fatty acid β-oxidation an imbalance between glucose oxidation and glycolysis leads to a net production of H^+ from the hydrolysis of glycolytically derived ATP (Opie, 1990; Lopaschuk et al., 1993). The excess production of H^+ leads to an impaired post-ischemic mechanical function in normal hearts (Lopaschuk et al., 1993). Furthermore, improvement in post-ischemic reperfusion recovery of normal hearts can be achieved by increasing the rates of glucose oxidation (McVeigh and Lopaschuk, 1990; Broderick et al., 1993).

With rates of glycolysis substantially increased above rates of glucose oxidation in the hypertrophied heart, an even greater imbalance between glucose oxidation and glycolysis exists. It may be that significantly increased rates of H^+ production in the hypertrophied heart during normal aerobic perfusion, and enhanced rates of H^+ production during post-ischemic reperfusion results in a H^+ overload which must be dealt with by the hypertrophied heart. The Na^+/H^+ exchanger is one such mechanism that is involved in the clearance of H^+ during post-ischemic reperfusion (Tani and Neely, 1989; Tani 1990; Fliegel and Frohlich, 1993). Other processes includes the H^+/lactate co-transporter and CO_2 washout. Both Na^+/H^+ exchange and H^+/lactate co-transport have been shown to play a significant role in the equilibration of H^+ balance after ischemia and during respiratory acidosis (Vandenberg et al., 1993). The contribution of these processes to post-ischemic reperfusion recovery in the hypertrophied heart have not been studied, although they may play a significant role.

It has previously been demonstrated that the Na^+/H^+ exchanger is involved in post-ischemic injury due to a subsequent Ca^{2+} overload via Na^+/Ca^{2+} exchange (Tani and Neely, 1989; Tani, 1990). Since there is an enhanced H^+ production in the hypertrophied heart and a possible increase in the Na^+/H^+ antiporter content (Avkiran, 1994), the possibility exists for a greater Ca^{2+} overload during post-ischemic reperfusion. Subsequent Ca^{2+} overload may be exaggerated in the hypertrophied heart during early post-ischemic reperfusion due to deficiencies seen in the mechanisms responsible for the handling of intracellular Ca^{2+} (Gwathmey and Morgan, 1985; Bentivegan et al., 1991; Bailey and Houser, 1993). As well, acidosis is associated with a decrease in twitch tension during ischemia and hypoxia (Ricciardi et al., 1986; Lee and Allen, 1991).

However, acidosis itself does not influence the contractile apparatus differently in the hypertrophied heart compared to normal hearts (Mayoux et al., 1994). Therefore the excessive H^+ production may lead to alterations in ion balance requiring a greater proportion of the ATP produced to be used for the re-equilibration of ion balance. This inappropriate use of ATP for the maintenance of altered ion balance during post-ischemic reperfusion may potentially be squandering valuable ATP necessary for contractile function. Calculations of cardiac efficiency described as an indice of cardiac function (HR x PSP) by steady state ATP production (μmol ATP$\cdot$min$^{-1}\cdot$g dry wt^{-1}), supports this hypothesis. A dramatic decrease in the cardiac efficiency of hypertrophied hearts occurred during post-ischemic reperfusion, compared to pre-ischemic values (from 0.29 to 0.08 HR$\cdot$PSP$\cdot 10^{-3}\cdot$(μmol ATP$\cdot$min$^{-1}\cdot$g dry wt^{-1})$^{-1}$).

Therefore, altered rates of glycolysis in the hypertrophied heart may lead to an elevated intracellular Na^+ and a subsequent Ca^{2+} overload due to increased Na^+/H^+ and Na^+/Ca^{2+} exchange (Tani and Neely, 1989; Tani, 1990). In an attempt to maintain ion balance, the hypertrophied heart has to expend ATP that is normally required for contractile function. The data supplied in this thesis show that the hypertrophied heart is not deficient in the production of ATP during post-ischemic reperfusion as seen by rates of glycolysis and oxidative metabolism that return to above pre-ischemic levels. Thus it may be that the use of ATP for membrane ion balance and not contractile function, leads to the depressed post-ischemic mechanical function seen in hypertrophied hearts compared to normal hearts.

The model of hypertrophy that has been used for the ischemia/reperfusion study, did not present a depression of pre-ischemic mechanical function compared to normal hearts. This may be due to an ample supply of exogenous energy substrate, i.e., the high exogenous palmitate concentration for energy production. As a result adequate ATP may be formed to allow for sustainable contractile function. The lack of mechanical depression may therefore depend on the availability of exogenous fatty acids (Table 3-2 compared to Figure 5-1), as well as the degree of hypertrophy (El Alaoui-Talibi et al., 1992). Cheikh et al. (1994) have recently suggested that the volume-overloaded hypertrophied rat heart is actually substrate limited especially when the primary source of exogenous carbon is a long-chain fatty acid (i.e., palmitate). In the model of hypertrophy presented here, it is not likely that there is a substrate limitation. In fact, the data indicate that the hypertrophied heart produces similar amounts of ATP from exogenous palmitate, compared to control hearts during both the pre- and post-ischemic periods. The fact that the hypertrophied heart is not as efficient compared to control hearts, especially during post-ischemic reperfusion may be the cause behind post-ischemic functional depression. The cause for an impaired efficiency in the hypertrophied heart may be due to the inappropriate use of ATP for the maintenance and re-establishment of membrane ion gradients, when ATP should be used for the recovery of contractile function. This hypothesis would agree with a recent study by Hata et al., (1994) who have shown that normal post-acidotic myocardium requires a greater VO_2 for non-mechanical activities per unit increase in E_{MAX}, suggesting an increased requirement for ATP to handle the excess H^+, and the ionic consequences of the acid load.

It can clearly be seen that both rates of glycolysis and oxidative metabolism recover fully during post-ischemic reperfusion of hypertrophied rat hearts. Accelerated rates of glycolysis and an exaggerated uncoupling of glycolysis from glucose oxidation leads to increased rates of H^+ production in the hypertrophied heart. The increased rates of H^+ production may therefore lead to disturbances in ion balance due to increased Na^+/H^+ and Na^+/Ca^{2+} transporter activities. Therefore alterations in Ca^{2+} handling in the hypertrophied heart may result in an inappropriate use of large amounts of ATP for maintenance of membrane ion gradients occurs. This in turn could deprive the heart of valuable ATP needed for the restoration of mechanical function, and the hypertrophied heart therefore fails to recover mechanical function.

D) Increasing glucose oxidation in the hypertrophied rat heart, increases aerobic mechanical function

A number of studies have demonstrated that PLC can improve contractile function in hypertrophied hearts (Mottorlini et al., 1992; Yang et al., 1992; Michelletti et al., 1994ab). This has been attributed, in part, to the ability of PLC to replenish myocardial L-carnitine stores, which are significantly depressed in the hypertrophied heart (Reibel et al., 1983; El-Alaoui-Talibi et al., 1992; Motterlini et al., 1992; Yang et al., 1992; Cheikh et al., 1994; Michelletti et al., 1994ab; Table 3-4; Figure 6-1). Decreased myocardial L-carnitine has been suggested to be responsible for the depression of fatty acid oxidation observed in the hypertrophied heart (El Alaoui-Talibi et al., 1992; Michelletti et al., 1994b). Data presented in this thesis show that a decrease in myocardial L-carnitine content exists, even though hearts were only mildly hypertrophied (Table 3-4; Figure 6-1). However, despite the fact that the decrease in L-carnitine was of the same magnitude as what has been observed in more severely hypertrophied hearts (El Alaoui-Talibi et al., 1992), no decrease in fatty acid oxidation rates occurred in hypertrophied hearts when the exogenous concentration of free fatty acids was high (i.e., 1.2 mM palmitate) compared to when it was low (i.e., 0.4 mM palmitate) (Figure 5-7 compared to Figure 3-3). Rather, the main alteration found in energy substrate metabolism of hypertrophied hearts remains to be an increase in glycolysis (Figure 3-1 and 5-8).

Acute PLC treatment was effective in completely reversing the decrease in L-carnitine seen in the hypertrophied hearts. Interestingly, the primary effect of PLC treatment was not to increase fatty acid oxidation, but rather to increase carbohydrate oxidation. This increase in carbohydrate oxidation (Figure 6-3) was accompanied by an increase in overall ATP production (Figure 6-6) and an increase in contractile function in the hypertrophied hearts (Figure 6-2). The primary effect of PLC was to increase the contribution of glucose oxidation to ATP production, and decrease the contribution of palmitate oxidation to ATP production. These observations suggest that the beneficial effects of PLC in hypertrophied hearts are primarily the result of its ability to stimulate carbohydrate oxidation. Chronic administration of PLC ($60\ mg \cdot kg^{-1} \cdot day^{-1}$) has also been shown to be effective in increasing myocardial L-carnitine content and improving cardiac function in hypertrophied hearts (Schönekess et al., 1995b).

L-carnitine is an important co-factor necessary for the oxidation of fatty acids.

CPT I, a key enzyme involved in fatty acyl-CoA uptake by the mitochondria, has an absolute requirement for L-carnitine. For this reason, a decrease in tissue L-carnitine has the potential to decrease fatty acid oxidation by limiting acyl CoA transport into the mitochondria. Recently, L-carnitine has also been recognized as having an important role in regulating carbohydrate oxidation (Broderick et al., 1992, 1993). This is thought to occur secondary to a stimulation of inner mitochondrial carnitine acetyltransferase (Lysiak et al., 1988). This enzyme transfers acetyl groups from mitochondrial acetyl-CoA to cytoplasmic acetylcarnitine resulting in a decrease in the inner mitochondrial acetyl-CoA/CoA ratio. A decrease in this ratio will stimulate PDH, the enzyme that converts pyruvate to acetyl-CoA, and is the rate limiting step of glucose oxidation. In support of this conclusion, it has previously been demonstrated that increasing L-carnitine levels in normal and diabetic hearts will increase the rate of glucose oxidation in fatty acid perfused hearts (Broderick et al., 1992, 1995b). As shown in Figure 6-3, PLC was also effective in stimulating glucose oxidation in both control and hypertrophied hearts. This probably occurred secondary to its effect of increasing L-carnitine levels and stimulating PDC in these hearts. As a result, a greater proportion of the pyruvate derived from glycolysis (glucose oxidation) as well as pyruvate derived from lactate (lactate oxidation) was oxidized.

The effects of PLC on energy metabolism may also have occurred secondary to replenishing TCA cycle intermediates, thereby increasing overall TCA cycle activity. PLC is an effective anaplerotic substrate (Davies et al., 1980; Di Lisa et al., 1989; Tasani et al., 1994), with the propionyl group being used to synthesize succinyl-CoA.

PLC did increase overall ATP production rates (and therefore overall TCA cycle activity) in the hypertrophied hearts. Whether an increase in TCA cycle activity is responsible for an increase in cardiac work or *visa versa* cannot be unequivocally concluded.

The relationship between myocardial L-carnitine levels and either fatty acid oxidation or carbohydrate oxidation has not been clearly delineated. In severe L-carnitine deficiencies, a depression in fatty acid oxidation occurs that can compromise muscle function. Treatment of rats with Na^+ pivalate also results in a marked decrease in myocardial L-carnitine content, with a parallel decrease in fatty acid oxidation rates (Broderick et al., 1995a). While it is clear that tissue levels of L-carnitine are decreased in the hypertrophied heart, it is not clear if this is responsible for a decrease in fatty acid oxidation. A study by El Alaoui-Talibi et al. (1992) and data presented in this thesis have shown that fatty acid oxidation rates can be depressed in isolated working hearts from volume-overloaded or aortic-banded rats, respectively. However, the reduction of fatty acid oxidation was most obvious at low-workloads in the presence of low concentrations of free fatty acids in pressure overloaded hypertrophied rat hearts (Figure 3-3). Fatty acid oxidation was not reduced in hypertrophied hearts subjected to increasing cardiac work, or in the presence of higher concentrations of free fatty acids (Figure 5-8). Thus, it does not appear that decreased fatty acid oxidation rates are limiting contractile function in the mildly hypertrophied heart. This may not be true in the severely hypertrophied hearts, however, where Cheikh et al. (1994) have shown that supplying these hearts with a carbon substrate that bypasses CPT 1 (i.e., octanoate) can improve myocardial energetics.

The stimulation of carbohydrate oxidation is the primary metabolic effect of L-carnitine or PLC supplementation in either normal hearts (Broderick et al., 1992) or in L-carnitine deficient hypertrophied hearts. PLC treatment markedly increased overall ATP production and the contribution of carbohydrate oxidation to ATP production. It may be that a limitation of CPT 1 activity and fatty acid oxidation would not likely occur until a severe L-carnitine deficiency occurs. Paradoxically, L-carnitine deficiencies severe enough to inhibit fatty acid oxidation may actually increase carbohydrate oxidation. In this setting, the decrease in mitochondrial acetyl-CoA/CoA ratio that would occur secondary to a decrease in β-oxidation would relieve the inhibition of PDC. In support of this concept, it has been demonstrated that the severe L-carnitine deficiency that occurs in hearts of Na^+ pivalate-fed rats is accompanied by an decrease in fatty acid oxidation, and an increase in glucose oxidation (Broderick et al., 1995a).

It has been observed that a significant increase in glycolysis in hypertrophied hearts compared to control hearts exists even in the presence of high circulating levels of exogenous free fatty acids. This increase in glycolysis, which is also seen in hypertrophied hearts perfused with low concentrations of free fatty acids, is consistent with the increase in enzyme activities associated with glycolysis (Bishop and Aultschuld, 1971; Taegtmeyer and Overturf, 1988). Despite this increase in glycolysis, rates of glucose oxidation were decreased in the hypertrophied hearts compared to normal hearts. These metabolic alterations create a dramatic uncoupling of glycolysis from glucose oxidation, resulting in a greater amount of pyruvate derived from glycolysis being converted to lactate. Uncoupling of glycolysis from glucose oxidation is a major source of H^+ production in the heart (Opie, 1990; Lopaschuk et al., 1993). Hydrolysis of ATP from each molecule of glucose that passes through glycolysis but not glucose oxidation produces 2 H^+ molecules (Figure 5-9). In contrast, glycolysis coupled to glucose oxidation is a H^+ neutral process (Opie, 1990). The excess production of H^+ due to an exaggerated uncoupling of glycolysis from glucose oxidation may contribute to contractile dysfunction in the hypertrophied heart. Recently, Hata et al. (1994) demonstrated that H^+ accumulation in the myocardium can markedly decrease cardiac efficiency. They postulate that this most likely occurs secondary to a stimulation of Na^+/H^+-exchange activity, with a subsequent increase in Na^+/Ca^{2+}-exchange activity. The accumulation of Na^+ and Ca^{2+} that occurs secondary to clearance of H^+ requires that a greater amount of ATP be directed towards basal metabolism, as opposed to contractile function. If the same situation is occurring in the hypertrophied heart because of an uncoupling of glycolysis and glucose oxidation, then stimulation of glucose oxidation may partly explain the benefit of PLC. In this regard, it has been demonstrated that cardiac efficiency is improved by chronic PLC administration to aortic-banded rats (Schönekess et al, 1995b). As a result, PLC has the potential to not only increase overall ATP production, but also to improve the efficiency of translating this ATP into contractile function.

The addition of L-carnitine to normal hearts has previously been shown to increase rates of glucose oxidation (Broderick et al., 1992), and improve post-ischemic reperfusion recovery (Broderick et al., 1993). PLC has also been shown to be beneficial in normal and hypertrophied hearts, and in human subject during post-ischemic reperfusion studies and during angina (Table 1-1 and 1-3). When 1 mM PLC was loaded into normal and hypertrophied rat hearts, it was found that rates of glucose oxidation increased dramatically during both pre- and post-ischemic perfusion periods (Figure 6-3 and 6-8). However, this did not dramatically improve mechanical function of either normal or hypertrophied hearts. One explanation may involve the continual accelerated production of H^+ by both normal and hypertrophied hearts (Figure 6-9). In fact, in normal hearts this significant increase in H^+ production may be due to an effect based on the Ca^{2+} content of the perfusate. In the study by Broderick et al. (1993), perfusate Ca^+ was 1.25 mM, and there was no acceleration of glycolytic rates during post-ischemic reperfusion in the L-carnitine treated hearts. However, in the experiments based in this thesis perfusate Ca^{2+} was set at 2.50 mM for the studies involving ischemia and reperfusion. In this setting, rates of glycolysis significantly increase during post-ischemic reperfusion when hearts are pretreated with PLC. However, numerous other studies have shown that PLC is beneficial during ischemia and reperfusion (Paulson et al., 1986, 1992; Liedtke et al., 1988). Therefore, the implication that the increased rates of glycolysis uncoupled from glucose oxidation are detrimental to post-ischemic reperfusion, are strengthened by this finding.

Both acute and chronic PLC treatment significantly increase cardiac work in mildly hypertrophied hearts (**CHAPTER VI**; Schönekess et al., 1995b). This benefit of PLC treatment is not related to an increase the contribution of fatty acid oxidation as a source of ATP production. Rather, the primary effect of PLC is to increase the contribution of carbohydrate oxidation to ATP production, presumably as a consequence of a reduction in mitochondrial acetyl-CoA/CoA ratio brought about by stimulation on carnitine acetyltransferase. This suggests that inner mitochondrial carnitine acetyltransferase may be an important target for improving energy substrate metabolism in the hypertrophied heart.

E) The contribution of glycogen and exogenous glucose to total rates of glycolysis and glucose oxidation during low-flow ischemia

The aerobically perfused hypertrophied heart has increased rates of glycolysis above those seen in normal hearts (Figure 3-1 and 5-2). This increase in glycolysis occurs even when glycogen synthesis is occurring (Figure 7-3), and it has been found that the rates of glycolysis are comparable to what has previously been shown to exist (Figure 3-1 and 5-2). The increase in glycolysis may be linked to the upregulation of some key glycolytic enzymes (Bishop and Aultschuld, 1971; Ingwall, 1984) or it may be an attempt to normalize ATP production. During ischemia, it has been suggested that the hypertrophied heart has a decreased ability to recruit anaerobic glycolysis and this may be detrimental during post-ischemic reperfusion (Cunningham et al., 1990; Gaasch et al., 1990). Other studies have suggested that the hypertrophied heart actually has accelerated rates of glycolysis during ischemia (Anderson et al., 1990a; Allard et al., 1994ab). The source of glucose during ischemia may be exogenous glucose if a residual coronary flow exists, but endogenous glycogen can also significantly contribute glucose for glycolysis (Cornblath et al., 1963). The exact contribution of glycogen to energy metabolism during ischemia is not definitively known in the hypertrophied heart.

The contribution of both endogenous glycogen and exogenous glucose during a low-flow (0.5 ml·min^{-1}) ischemia to rates of glycolysis and glucose oxidation was previously unknown. It was found that rates of glycolysis during ischemia are no longer increased in hypertrophied hearts, as they are during aerobic perfusion (Figure 7-3). It is also evident that there is a significant glycogen component to total rates of glycolysis, that can readily be measured by the model of glycogen depletion and resynthesis developed in this thesis, in both control and hypertrophied hearts. Glycogen's contribution to glycolysis during low-flow ischemia may be slightly accelerated in the hypertrophied heart compared to normal hearts, comprising 33% and 24% respectively of the total glycolytic rates during low-flow ischemia. However, during a low-flow ischemia the hypertrophied heart does not have increased rates of glycolysis beyond that of a normal heart.

Rates of glucose oxidation were also measured in the control and hypertrophied hearts during aerobic perfusion and low-flow ischemia. It was found that rates of glucose oxidation during low-flow ischemia were significantly higher than what has previously seen in control and hypertrophied hearts perfused under similar buffer conditions (Figure 5-4). This may have been due to the decreased contribution of glycogen to glucose oxidation. The model of low-flow ischemia that we used effectively abolished mechanical function, and resulted in a severely reduced oxidative metabolism. Rates of glucose oxidation from exogenous glucose dropped dramatically compared to the pre-ischemia values (Figure 7-5). However, glycogen metabolism played a significant role in supplying residual oxidative metabolism with glucose for glucose oxidation.

The data indicates that nearly 20 % of the glucose passing through glycolysis in controls and 15 % in hypertrophied hearts goes to residual oxidative metabolism. This compares to 6 % of exogenous glucose passing through glycolysis going to residual glucose oxidation for both control and hypertrophied hearts during ischemia. This preferential usage of glucose from glycogen is not unlike what has previously been shown in carotid artery smooth muscle (Hardin et al., 1994). Hardin *et al.*, (1994) suggest that in smooth muscle, glycogen derived pyruvate passes preferentially into glucose oxidation. Recently it has also been shown that during aerobic perfusion of isolated working rat hearts, a preferential use of glucose from glycogen for glucose oxidation exists (Henning et al., 1995). This finding strengthens the implications made by Hardin et al, (1994) who suggested that pyruvate formed from exogenous glucose via glycolysis and pyruvate from glycogen metabolism, do not form a homogenous intracellular pyruvate pool. Lynch and Paul (1987) have found that in vascular smooth muscle the use of glycogen for oxidative metabolism correlated with contractile activity, whereas glucose uptake favoured aerobic glycolysis. Therefore it is possible that the glycogen pool in the heart is also favoured for oxidative metabolism and contractile function and aerobic glycolysis from exogenous glucose may be used preferentially for sarcoplasmic reticulam Ca^{2+} uptake and membrane ion balance. This division of glycolytic pathways and pyruvate formation would support a model of compartmentalization.

As well, it is interesting to note that rates of glucose oxidation increased over the 60 min resynthesis period in both control and hypertrophied hearts. This may be due to a greater cycling of glucose from glycogen into glucose oxidation as the glycogen pool increased in size (and is ^{14}C-labelled) during resynthesis. Further evidence for this is based on the enhanced rates of glucose oxidation seen in both control and hypertrophied hearts during the aerobic resynthesis period. Compared to hearts perfused under similar conditions (Figure 5-4), rates of glucose oxidation are 4 to 5-fold greater in hearts undergoing glycogen resynthesis (Figure 7-5). This suggests that greater amounts of pyruvate from exogenous glucose passing through glycolysis are proceeding to glucose oxidation. Why this occurs may be due to the depleted glycogen pool, which because it is being resynthesized, may not contribute to glucose oxidation to such a great extent. As the glycogen pool increases in size the increased rates of glucose oxidation at the end of the 60 min aerobic period may come from the increased turnover of ^{14}C labelled glycogen, and its preferential support of glucose oxidation.

Depletion of myocardial glycogen stores was not significantly different between hypertrophied hearts and control hearts at the end of the low-flow ischemic period (Table 7-3). This supports data in which glycogen breakdown was directly measured with radiolabelled glucose. Rates of glycolysis from glycogen and exogenous glucose are linear during low-flow ischemia. The residual labelling of the glycogen pool at the end of low-flow ischemia indicates that the radiolabel was not totally removed from the glycogen pool. The fact that the glycogen pool at the end of low-flow ischemia was labelled to a similar extent as at the end of the resynthesis period, suggests that the labelled glucose units of the glycogen pool were not uniformly metabolised. The radiolabelling data also indicate that there may be a minor component of glycogen resynthesis during the low-flow period. At the end of low-flow ischemia, 16.1 ± 1.8 % of the glycogen pool in control hearts was labelled with the radioisotope of glucose that was present only during low-flow ischemia. A value of 24.1 ± 3.3 % of the glycogen pool in hypertrophied hearts was labelled with the radioisotope of glucose present only during low-flow ischemia. It is clearly evident that the glycogen phosphorylase reaction is greatly exceeding the glycogen synthase reaction because a net glycogen breakdown does occur, and there was a measurable difference in the rates of total glycolysis and rates of glycolysis from exogenous glucose. However this data implicates a simultaneous glycogen breakdown and resynthesis during low-flow ischemia.

Elevated rates of glycolysis compared to rates of glucose oxidation have been linked to depressed post-ischemic functional recovery of normal hearts (Lopaschuk et al., 1993). Uncoupling glucose oxidation from glycolysis leads to H^+ production (Opie, 1990; Dennis et al., 1991; Lopaschuk et al., 1993). This uncoupling occurs in the aerobically perfused heart and is due to the inhibition of the PDH complex (PDC) by a high intramitochondrial acetyl-CoA/CoA ratio produced by the preferred oxidation of fatty acids (Patel and Roche, 1990; Saddik and Lopaschuk, 1991). Therefore, due to the inhibition of PDC by fatty acid ß-oxidation an imbalance between glucose oxidation and glycolysis leads to a net production of H^+ from the hydrolysis of glycolytically derived ATP (Opie, 1990; Dennis et al., 1991; Lopaschuk et al., 1993). Although the study on glycogen turnover did not address the recovery of mechanical function, **CHAPTER V** implicates an excess production of H^+ as a cause for an impaired post-ischemic mechanical function in hypertrophied hearts compared to normal hearts (Figure 5-1).

Evidence presented in this thesis suggests that an accelerated rate of glycolysis in the hypertrophied heart prior to and following ischemia results in an increased rate of H^+ production due to enhanced rates of glycolysis, as well as glycolytic metabolite accumulation (Bishop and Aultschuld, 1971; Anderson et al., 1990a; Allard et al., 1994a). During a low-flow ischemia this may not be the case. Rates of glycolysis and residual oxidative metabolism from exogenous sources and glycogen were similar in the hypertrophied heart compared to normal hearts. This should result in a similar rate of ATP production and net H^+ production from the hydrolysis of glycolytically derived ATP (Opie, 1990; Dennis et al., 1991). Rates of H^+ production from both endogenous glycogen and exogenous glucose during low-flow ischemia yield 7.116 ± 1.097 *vs* 8.202 ± 2.255 $\mu mol \cdot min^{-1} \cdot g$ dry wt^{-1} for control and hypertrophied hearts, respectively. These similar rates of H^+ production do not support the conclusions drawn from data concerning tissue lactate levels, which suggest a greater metabolism of glucose due to increased lactate accumulation (Table 7-3). The significant accumulation of lactate in the hypertrophied heart compared to controls at the end of low-flow ischemia, was not unlike what was seen at the end of the aerobic resynthesis period, where the hypertrophied heart had a greater tissue lactate content (Table 7-3). However, in the model of low-flow ischemia used there was a lower residual coronary flow rate in the hypertrophied heart on a per gram tissue basis due to the greater mass of the hypertrophied heart. Although the accumulation of lactate suggests a greater rate of glycolysis, it was clear from the direct measurement of glucose breakdown and from the measures of tissue glycogen depletion, that glycolysis was not accelerated in the hypertrophied heart during low-flow ischemia. During the glycogen resynthesis period, the hypertrophied heart had significantly increased rates of glycolysis, which may have contributes to the accumulation of tissue lactate. Evidence against an actual hypoxia in the hypertrophied heart during this period came from the robust rates of glucose oxidation, which were similar to what was seen in the control hearts, and in fact accelerated above what we have previously seen (Figure 5-4).

Substantially increased rates of glycolysis above rates of glucose oxidation in the hypertrophied heart result in a significantly increased production of H^+ during normal aerobic perfusion and during post-ischemic reperfusion. It has been shown that the Na^+/H^+ exchanger message (NHE-1) is increased in the hypertrophied heart (Avkiran, 1994), suggesting that the hypertrophied heart may be upregulating systems that are used to handle the acidosis produced by enhanced glycolytic rates. The Na^+/H^+ exchanger may be involved in post-ischemic injury due to subsequent Ca^{2+} overload from Na^+/Ca^{2+} exchange (Tani and Neely, 1989; du Toit and Opie, 1993). Therefore increased rates of H^+ production in the hypertrophied heart due to accelerated rates of glycolysis during ischemia may result in an exacerbated Na^+/H^+ and Na^+/Ca^{2+} activity and subsequent Ca^{2+} overload during early reperfusion. The contribution of ischemically derived H^+ to post-ischemic reperfusion injury was not increased in the hypertrophied heart above normal hearts during low-flow ischemia. However, what occurs during a global ischemia may be different, as suggested by a number of other studies (Bishop and Aultschuld, 1971; Anderson et al., 1990a; Allard et al., 1994ab). The present study was limited to low-flow ischemia, so that metabolism could be measured by the washout of glycolytic metabolites (^3H_2O), which is not possible during global ischemia. The recovery of mechanical function after low-flow ischemia in hypertrophied hearts was not measured in this study, therefore it can not be stated for certain if the rates of H^+ production in hypertrophied hearts during low-flow ischemia affects post-ischemic mechanical function.

The model of glycogen depletion, resynthesis and labelling is a good model for following the fate of glycogen during ischemia. It was found that total rates of glycolysis are not enhanced in the hypertrophied heart during low-flow ischemia. Similarly, rates of exogenous glucose use are not accelerated in the hypertrophied heart compared to control hearts, unlike what is seen during normal aerobic perfusion. Glycogen contributes a significant percentage to total rates of glycolysis during low-flow ischemia. Since ischemic glycolytic rates are not significantly different in hypertrophied hearts, the contribution of glycolysis during ischemia may not play a conspicuous role in the depression of post-ischemic reperfusion recovery of hypertrophied hearts, beyond what is seen in normal hearts.

F) Methodological considerations

i) Mechanical function

None of the studies in this thesis was designed to compare directly differences in contractility between normal and hypertrophied hearts. The working heart preparation was used since hearts can perform considerable work in this model (de Leiris et al.,

1984) and can achieve significantly greater levels of oxygen consumption than isovolumic, Langendorff perfused hearts (Morgan et al., 1984). The product of HR and PSP was used to quantitate left ventricular function as an index of cardiac function, as was CO and PSP when CO was measured in the hearts. Determination of other indices of myocardial work, such as systolic wall stress, would have provided a more sensitive index of myocardial energy expenditure. Since the rate-pressure product has been shown to have a strong relationship to myocardial oxygen consumption and metabolism (Neely et al., 1976), it was sufficient for the purposes of this thesis.

The work that the hypertrophied hearts produced in the isolated working heart mode was not matched for the pathophysiological situation that the heart was exposed to *in vivo* (i.e., an increased pressure load). There was also no attempt made to match the work produced in normal hearts and hypertrophied hearts relative to each other. This would have yielded a situation where the either the control or hypertrophied heart was performing an un-physiological amount of work. The attempt to match work to *in vivo* situations, would have made the process of measuring metabolism (which is the focus of these studies) difficult. The hearts would not be exposed to the same amount of external workload, and therefore any metabolic comparisons would have been difficult to make. Additionally, the studies designed in this thesis were designed to look at metabolism. It would be unreasonable to assume that all physiological parameters and conditions could be matched between the control and hypertrophied hearts, and the *in vivo* situation. Although it could be argued that the hypertrophied heart was actually working at a lower level compared to normal hearts, since the workloads were not necessarily matched to *in vivo* conditions, measurement of PSP and CW (when measured) showed similar workloads when the hearts were exposed to a similar external pressure-load.

ii) Degree of myocardial hypertrophy

The degree of myocardial hypertrophy produced by banding the abdominal aorta was mild. A 15-35% increase in myocardial mass produced by this method. This mild degree of hypertrophy resulted in only minor functional alterations during normal aerobic perfusion, which could be reversed upon supplying the hypertrophied hearts with higher amounts of exogenous fatty acids. However, mechanical depression seen in hypertrophied hearts during post-ischemic reperfusion was apparent in this model of hypertrophy even though the increase in myocardial mass was only 15%. Metabolic abnormalities were consistent in this model of hypertrophy, in that the hypertrophied hearts had decreased levels of tissue L-carnitine, similar to what is seen in more severe models of hypertrophy (El Alaoui-Talibi et al., 1992). The major metabolic finding of increased rates of glycolysis was consistent throughout all perfusion protocols in this thesis.

iii) Measurements of myocardial metabolism

A limitation of measuring substrate use in this thesis, is that endogenous contributions (triacylglycerols and glycogen) to energy substrate metabolism were not made (except for the low-flow ischemia study in which glycogen metabolism was measured). The study done at 0.4 mM palmitate **(CHAPTER III)** suggests that an unmeasured substrate may significantly contribute to overall ATP production in hypertrophied hearts. This important issue, has been addressed in normal hearts, and indeed, at 0.4 mM palmitate endogenous triacylglycerol does contribute significantly to ATP production (Saddik and Lopaschuk, 1991). However, knowing that there is a significant mechanical dysfunction associated with myocardial hypertrophy, studies progressed to ischemia and reperfusion, and once the identification of altered glycolytic rates was made as a key alteration in the hypertrophied heart, the contribution of endogenous triacylglycerols to ATP production was not investigated. However, this is an area that does need to be investigated considering fatty acid utilization can be impaired in the hypertrophied hearts. Endogenous glycogen during ischemia was measured since the metabolism of glycogen is a major source of ATP during ischemia via the glycolytic pathway. A drawback of the glycogen protocols is that a low-flow ischemia was necessary to measure metabolism, since it was necessary to have the glycolytic and oxidative metabolites washed out in the coronary effluent. However it is justifiable to consider the low-flow model and the global ischemia model as representative models, because both situations are physiologically relevant models.

Many studies looking at glycolytic metabolism in normal and hypertrophied hearts have looked at the production of lactate as an indication of glycolytic flux. The major drawback to this method is that this does not allow any conclusions to be made about glucose oxidation, which can vary dramatically compared to glycolysis depending on the perfusion conditions that are being used, and effect the amount of lactate that is being formed. The methodology employed in this thesis involved directly measuring glycolysis and glucose oxidation, by measuring the production of glycolytic and oxidative products (^3H_2O and $^{14}CO_2$, respectively). This method is sometimes compared to studies in which lactate release is measured. However as stated above, lactate release is an insensitive measure and only relates the difference between glycolysis and glucose oxidation.

To investigate the relationship between measuring glucose metabolism by the indirect method of quantifying lactate release and the direct method of measuring glycolytic and oxidative metabolite formation, measurements of lactate release during normal aerobic perfusion **(CHAPTER VII)** were made. This involved the separation of radiolabelled [U-^{14}C] lactate produced from the glycolytic breakdown of [U-^{14}C] glucose, from labelled glucose. The methodology for this is explained in **CHAPTER II**. The results suggest that under 50% of the label actually being released in normal hearts could be accounted for compared to what is directly measured. This casts doubt on measurements of lactate release as a valuable measure of glucose metabolism.

G) Conclusion

It is the contention of this thesis that the primary metabolic alteration that exists in the pressure-overloaded hypertrophied heart is an increase in glycolytic rate. This leads to a greater uncoupling of glycolysis from glucose oxidation, and an increased production of H^+ from glucose metabolism. PLC treatment has the potential to improve the coupling between myocardial glycolysis and glucose oxidation, resulting in a decrease in H^+ production derived from glucose metabolism. During ischemia, glycolysis and glycogen utilization are not altered in the hypertrophied heart compared to normal hearts. There is not a depressed ability in the hypertrophied heart to recruit glycolysis or oxidative metabolism during post-ischemic reperfusion. A decreased post-ischemic functional recovery in hypertrophied hearts may be linked the increased acidification of the hypertrophied myocyte and the increased use of ATP for non-contractile purposes, such as the maintenance of membrane ion balance.

CHAPTER IX

Future Directions

From the data revealed in this thesis, there are many questions that remain to be answered relating to energy metabolism in the hypertrophied heart, and the mechanical consequences of an altered energy metabolism. Some of these questions could lead to valuable information concerning the metabolism of energy substrates in the heart.

A) Contribution of endogenous triacylglycerol to overall ATP production in the hypertrophied heart

From the data in **CHAPTER III** the question arises as to why the hypertrophied heart has a decreased total rate of ATP production as measured from exogenous sources? As discussed this may be due to the contribution of endogenous triacylglycerols to overall ATP production. To examine this question, experiments similar to those done in normal hearts (Saddik and Lopaschuk, 1991) would have to be carried out. This would involve a series of isolated working heart perfusions where the endogenous lipid pool was "pulse-labelled", and then "chased" to measure the contribution of triacylglycerol to overall ATP production. It is known that in a state of 0.4 mM exogenous palmitate, triacylglycerol significantly contributes to ATP production in the isolated working rat heart. It would be of interest if this occurred in the hypertrophied heart, since there is also the notion that a decreased L-carnitine content will decrease fatty acid oxidation.

However, data in this thesis have shown that the decreased level of L-carnitine seen in hypertrophied hearts is potentially limiting for fatty acid oxidation only in situations of low-work and low exogenous fatty acid concentration (i.e., 0.4 mM palmitate).

B) Improvement of post-ischemic mechanical function by decreasing glycolysis

It has been shown that the hypertrophied heart has a greater rate of glycolysis than that seen in normal hearts. This leads to a greater rate of H^+ production due to the increased uncoupling of glycolysis from glucose oxidation. If the increased H^+ production is the cause of the depressed post-ischemic mechanical function seen in hypertrophied hearts compared to normal hearts, as is hypothesized in this thesis, then alteration of H^+ production or extrusion may be of benefit.

An attempt was made to improve the coupling of glycolysis to glucose oxidation in the hypertrophied heart during post-ischemic reperfusion (**CHAPTER VI**). Although PLC increased rates of glucose oxidation, there was no dramatic decrease in the production of H^+. Therefore, if the coupling of glycolysis to glucose oxidation can not be altered by increasing glucose oxidation, perhaps altering glycolysis will decrease H^+ production. Adenosine has been shown to decrease glycolysis in the isolated working rat heart (Finegan et al., 1992), and improve mechanical post-ischemic reperfusion recovery of normal hearts by decreasing glycolysis and potentially decreasing H^+ production (Finegan et al., 1993). Whether or not adenosine will decrease the rates of glycolysis seen in the hypertrophied heart is not known.

C) Blockade of H^+ extrusion and subsequent Ca^{2+} overload by the Na^+/H^+ and Na^+/Ca^{2+} exchange system

The excessive production of H^+ by the hypertrophied heart can potentially lead to increased Ca^{2+} overload by an increased of the Na^+/H^+ and Na^+/Ca^{2+} exchanger activity. This may cause the greater depression of post-ischemic mechanical function seen in hypertrophied hearts compared to normal hearts. Blockade of the Na^+/H^+ exchanger by amilioride and amilioride analogues can potentially decrease the Ca^{2+} overload seen by an active Na^+/H^+ and Na^+/Ca^{2+} exchange system, and improve post-ischemic mechanical function in normal hearts (Tani and Neely, 1989; Fliegel et al., 1993). The potential effects of blocking H^+ extrusion in the hypertrophied heart may therefore be of benefit, since it has been shown that not only does the hypertrophied heart have increased rates of H^+ production, there is also evidence of an upregulation of the Na^+/H^+ exchanger system (Avkiran, 1994). A blockade of the Na^+/H^+ exchanger system may lead to a greater efflux of H^+ via the H^+/lactate co-transporter. This process does not affect Ca^{2+} balance, and therefore would be of benefit. However, the effects of amilioride and amilioride derivatives on the post-ischemic mechanical function and energy metabolism of hypertrophied hearts are not known.

CHAPTER X

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CHAPTER XI

Curriculum Vitae

Personal information

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Date of Birth:	May 12, 1969
Place of Birth:	Vancouver, British Columbia, Canada
Citizenship:	Canadian
Hobbies and Interests:	Rock climbing, back country backpacking, scuba diving, running, biking, reading, history.

Education

University of Alberta, Edmonton, Alberta, Canada; B.Sc., 1992. Specialization in Pharmacology

University of Alberta, Edmonton, Alberta, Canada; Ph.D., 1996. Pharmacology. Ph.D. Thesis title; Energy Metabolism in the Hypertrophied Heart.

Laboratory and teaching experience

10/90-04/91: Laboratory Technician/Student, Department of Pharmacology, University of Alberta. Supervisor: Dr. A. R. P. Paterson.

01/92-present: Graduate Student, Department of Pharmacology, University of Alberta. Supervisor: Dr. G. D. Lopaschuk.

01/94-05/94: Teaching Assistant-PMCOL 335 (52 hours): Advanced animal experimentation. Department of Pharmacology, University of Alberta. Course Director: Dr. G. Hunter.

03/94: Lecture on Congestive Heart Failure in PMCOL 415/515 (1 hour):

Cardiovascular Pharmacology. Department of Pharmacology, University of Alberta. Course Director: Dr. G. D. Lopaschuk.

01/95-05/95: Teaching Assistant-PMCOL 335 (100 hours): Advanced animal experimentation. Department of Pharmacology, University of Alberta. Course Director: Dr. G. Hunter.

Volunteer duties

05/93-05/94: Graduate Student Representative for the Department of Pharmacology, to the Graduate Students Association, University of Alberta.

09/94: Member of Graduate Students Association Focus Group on Federal Science & Technology Funding Policy: Requested by Dr. M. Piper, U of A VP Research.

05/94-04/95: Graduate Student Representative to the Department of Pharmacology, University of Alberta.

Distinctions and awards

1987: University of Alberta Matriculation Award.

1991: University of Alberta Undergraduate Award.

1992: Mary Louise Imrie Award.

1992-present: Alberta Heritage Foundation for Medical Research Studentship.

1994-present: Heart and Stroke Foundation of Canada Research Traineeship.

1996-1998: Alberta Heritage Foundation for Medical Research Post-doctoral Fellowship.

Publications

Papers (published, in press, submitted, and in preparation):

1) Allard, M. F., **Schönekess, B. O.**, Henning, S. L, English, D. R. and Lopaschuk, G. D. The Contribution of Oxidative Metabolism and Glycolysis to ATP Production in the Hypertrophied Heart. Am. J. Physiol. 267:H742-750 (1994).

2) Lopaschuk, G. D., Belke, D. D., Gamble, J., Itoi, T. and **Schönekess, B. O.** Regulation of Fatty Acid Oxidation in the Mammalian Heart in Health and Disease. Biochim. Biophys. Acta 1213: 263-276 (1994).

3) **Schönekess, B. O.**, Allard, M. F. and Lopaschuk, G. D. Propionyl L-Carnitine Improvement of Hypertrophied Heart Function is Accompanied by an Increase in Carbohydrate Oxidation. Circ. Res. 77:726-734.

4) **Schönekess, B. O.**, Allard, M. F. and Lopaschuk, G. D. Propionyl L-Carnitine Improvement of Hypertrophied Rat Heart Function is Associated with an Increase in Cardiac Efficiency. (in press Euro. J. Pharmacol.).

5) **Schönekess, B. O.**, Brindley P., and Lopaschuk, G. D. Calcium Regulation of Glycolysis and Glucose Oxidation in the Isolated Fatty Acid Perfused Heart. (in press Can. J. Physiol. Pharmacol.).

6) Henning, S. L., Wambolt, R. B., **Schönekess, B. O.**, Lopaschuk, G. D., and Allard, M. F. Contribution of Glycogen to Aerobic Myocardial Glucose Utilization. (in press Circulation).

7) **Schönekess, B. O.**, Allard, M. F. and Lopaschuk, G. D. Recovery of Glycolysis and Oxidative Metabolism During Post-Ischemic reperfusion of Hypertrophied Rat Hearts (accepted Am J Physiol).

8) **Schönekess, B. O.**, and Lopaschuk, G. D. Competition between lactate and fatty acids as sources of ATP in the heart. (in preparation).

9) **Schönekess, B. O.**, Allard, M. F, Henning, S. L., Wambolt, R. B., and Lopaschuk, G.D. Contribution of Glycogen and Exogenous Glucose to Glucose Metabolism During Low-Flow Ischemia in Pressure Overloaded Hypertrophied Rat Hearts. (in preparation).

Book chapters

1) **Schönekess, B. O.** and Lopaschuk, G. D. The Effects of Carnitine on Myocardial Carbohydrate Metabolism. In: The Carnitine System and the Heart, de Jong, J. W., Ferrari, R. (eds.), Kluwar Academic Publishers, Dordrecht/Boston/London. (1995).

2) **Schönekess, B. O.**, and Lopaschuk, G. D. Metabolic Derangement's in Pressure Overload Cardiomyopathies. In: Cardionephrology$_3$, Timio, M., Wizemann V., Venanzi S. (eds.), Editoriale Bios, Corsenze, Italy;1995:321-325.

Abstracts

1) **Schönekess, B. O.** and Lopaschuk, G. D. The Importance of Glycolysis Versus Glucose Oxidation on Rates of Mechanical Failure of Rat Hearts Subjected to Low-Flow Ischemia. J. Mol. Cell Cardiol. 24(suppl III):S21 (1992).

2) **Schönekess, B. O.**, Allard, M. F. and Lopaschuk, G. D. Myocardial Energy Substrate Use in Aerobically Perfused Pressure-Overloaded Hypertrophied Rat Heart. Eur. Heart J. 14(abstr. suppl):222 (1993).

3) Allard M. F., **Schönekess, B. O.**, Henning, S. L., English, D. R. and Lopaschuk, G. D. Myocardial Energy Substrate Use in Aerobically Perfused Pressure-Overloaded Hypertrophied Rat Heart. Can. J. Cardiol. 9(suppl E): 86E (1993).

4) **Schönekess, B. O.**, Allard, M. F. and Lopaschuk, G. D. Hydrogen Ion Production from Glucose Metabolism in Hypertrophied Rat Hearts. Can. J. Cardiol. 10(suppl A): 98A (1994).

5) **Schönekess, B. O.**, Allard, M. F., Kozak, R. M., Barr, R. L. and Lopaschuk G. D. L-Propionyl Carnitine Feeding Improves Hypertrophied Rat Heart Function. J. Mol. Cell. Cardiol. 26(7)CLXV (1994).

6) **Schönekess, B. O.**, Allard, M. F, and Lopaschuk, G. D. Improved Hypertrophied Rat Heart Function by L-Propionyl Carnitine is Accompanied by an Increase in Glucose Oxidation. Can. J. Physiol. Pharmacol. 72(suppl 1):102 (1994).

7) **Schönekess, B. O.** and Lopaschuk, G. D. Competition Between Lactate and Fatty Acid Oxidation as Sources of ATP Production in the Heart. Can. J. Cardiol. 10(suppl C):134C (1994).

8) **Schönekess, B. O.**, Allard, M. F, Henning, S. L., Wambolt, R. B., and Lopaschuk, G. D. Glycogen Utilization During Low-Flow Ischemia in Hypertrophied Rat Hearts. J. Mol. Cell. Cardiol. 27(5):A34 (1995).

9) Henning S. L., Wambolt, R. B., **Schönekess, B. O.**, Lopaschuk, G. D., and Allard M. F. Glucose Oxidation from Glycogen in Fatty-Acid Perfused Rat Hearts. J. Mol. Cell. Cardiol. 27(5):A35 (1995).

10) **Schönekess, B. O.** and Lopaschuk, G. D. Lactate and Fatty Acids as Sources of ATP Production in the Heart. J. Mol. Cell. Cardiol. 27(6):A174 (1995).

Oral Presentations

1) "Hydrogen Ion Overload in Hypertrophied Rat Hearts". Prassis Sigma Tau Research Institute. Milano, Italy. Sept. 6, 1993.

2) "Competition Between Lactate and Fatty Acid Oxidation as Sources of ATP Production in the Heart". Canadian Cardiovascular Society Meeting. Edmonton, Alberta. Oct. 29, 1994.

3) "Energy Metabolism in the Hypertrophied Heart". University of Alberta, Edmonton, Canada. Dec. 12, 1995.